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BULLETIN

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ENDOTOXINS AND GRAM-NEGATIVE INFECTIONS

A Symposium

IVAN L. BENNETT, JR.

ABRAHAM I. BRAUDE and JENNIE SIEMIENSKI

LUCIEN B. GUZE

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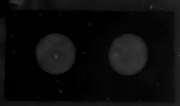

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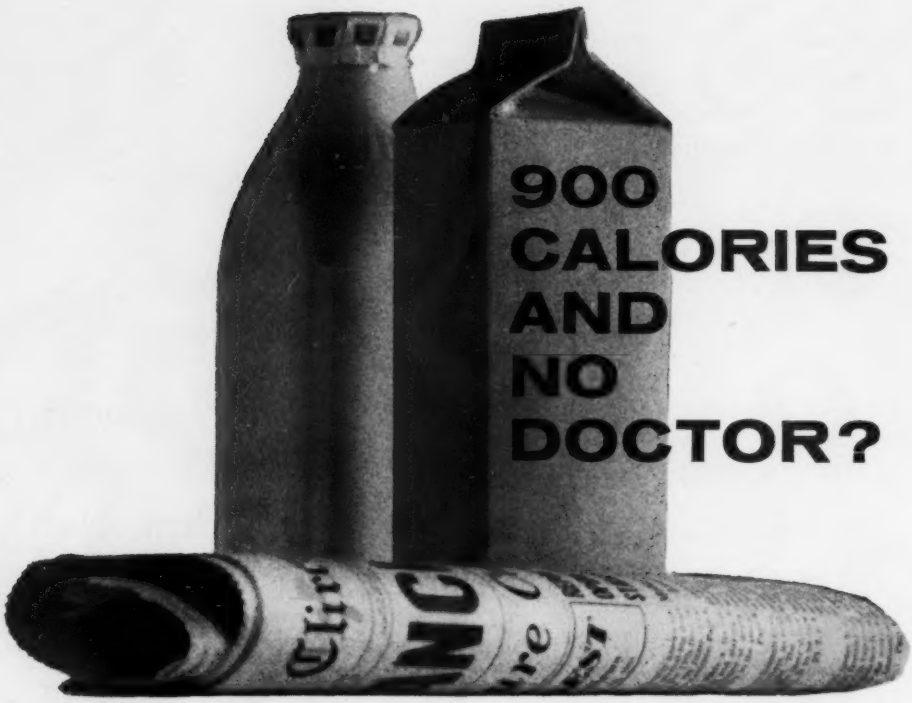

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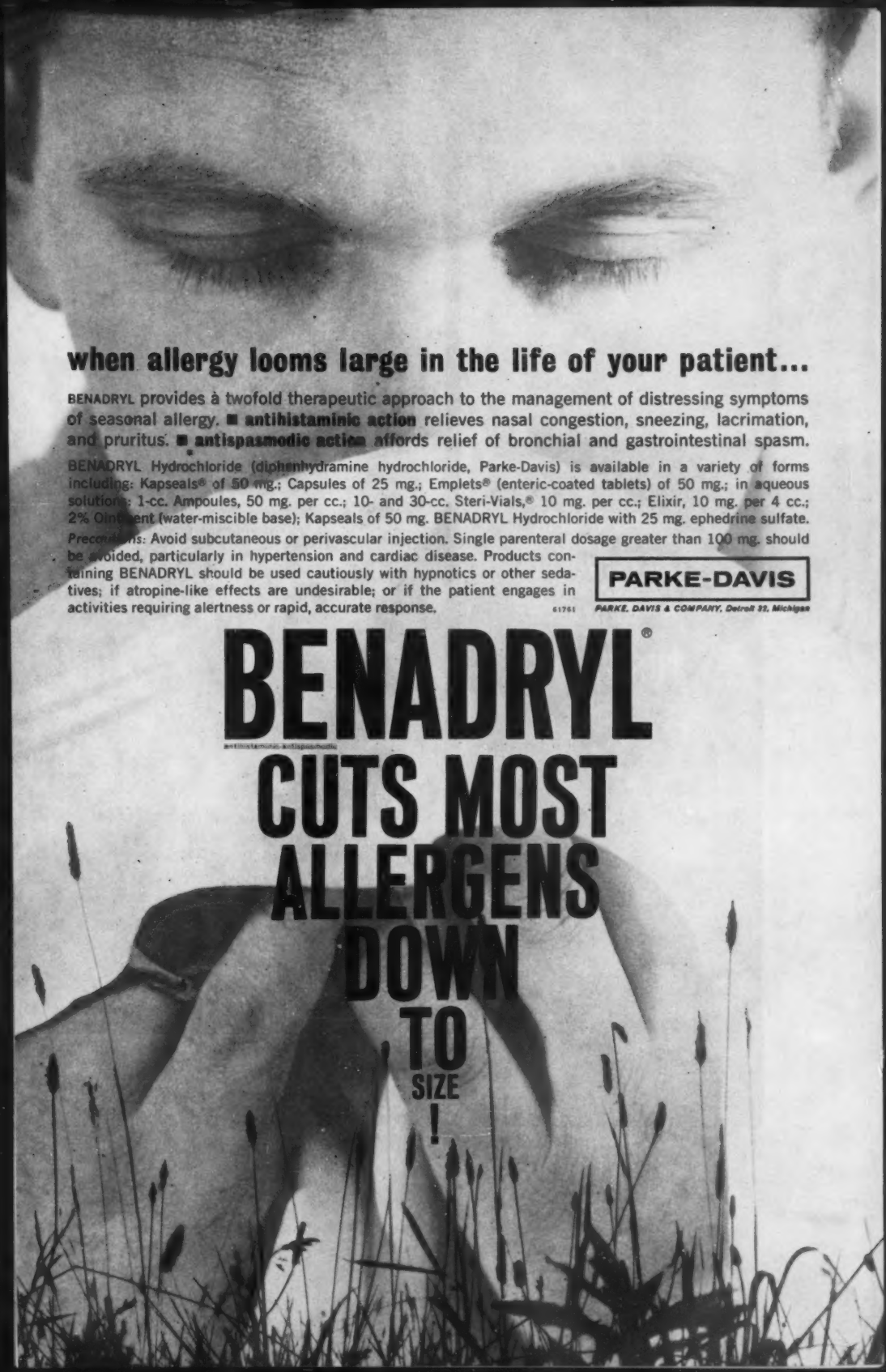

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1. Shlevin, E. L.: Pregnancy and Diabetes, Diabetes, 6:523, 1957.

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AUTHORS ALONE ARE RESPONSIBLE FOR OPINIONS EXPRESSED IN THEIR CONTRIBUTIONS

ROBERT L. CRAIG, *Editor*

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BULLETIN OF
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OF MEDICINE

VOL. 37, NO. 7 JULY 1961

Symposium on*

ENDOTOXINS AND GRAM-NEGATIVE INFECTIONS

1. PATHOGENESIS OF FEVER
IVAN L. BENNETT, JR., Johns Hopkins University School of Medicine

2. THE INFLUENCE OF ENDOTOXIN ON RESISTANCE TO INFECTION
ABRAHAM I. BRAUDE and JENNIE SIEMIENSKI, University of Pittsburgh School of Medicine

3. CONSIDERATION OF CERTAIN HOST FACTORS IN PYELONEPHRITIS
LUCIEN B. GUZE, Veterans Administration Hospital, Los Angeles

4. VASCULAR EFFECTS OF ENDOTOXINS
CHANDLER A. STETSON, JR., New York University School of Medicine

5. ROLE OF ENDOTOXIN IN TYPHOID FEVER
SHELDON E. GREISMAN, RICHARD B. HORNICK
THEODORE E. WOODWARD MERRILL J. SNYDER
University of Maryland School of Medicine

6. SALMONELLOSIS: CERTAIN FACTORS INFLUENCING THE INTERACTION OF SALMONELLA AND THE HUMAN HOST
EDWARD W. HOOK, Cornell University Medical School

* Held by the Section on Medicine, The New York Academy of Medicine, March 25, 1961. Carl Muschenheim, *Chairman*, Howard E. Eder, *Secretary*.

Supported by a Grant-in-Aid from the Merck Sharp and Dohme Postgraduate Program.

PATHOGENESIS OF FEVER

IVAN L. BENNETT, JR.

Professor and Chairman, Department of Pathology, The Johns Hopkins University School of Medicine and The Johns Hopkins Hospital, Baltimore, Maryland

IT HAS been known since the last century that the injection of Gram-negative bacteria or their products will produce fever in man.[1, 2] This procedure, using vaccines or "purified" endotoxins, continues to be a standard method for inducing therapeutic fever in human subjects. Several animals, including the dog, cat and rabbit, are also regularly susceptible to the pyrogenic action of endotoxin. Consequently, endotoxin fever has been the laboratory model for many studies of altered temperature regulation and a considerable amount has been learned. Indeed, endotoxin fever has come to be a sort of standard with which other forms of experimental fever are to be compared. Another reason for regarding endotoxin fever as a standard is the enormous frequency with which biological materials become contaminated by the pyrogenic products of Gram-negative bacteria. The avoidance of this contamination is a great practical problem in preparing materials for parenteral use in man. Furthermore, it is always necessary to rule out contamination by endotoxin when dealing with other materials of supposed pyrogenic activity in the laboratory.

The subject of this symposium is clearly stated to be *Endotoxins and Gram-Negative Infections*. In the interest of relevancy, this presentation will be limited to a discussion of endotoxin fever, although clarity will demand occasional brief references to other forms of experimental fever.

After a description of the febrile response to endotoxin, present knowledge of the mechanism of this type of fever will be summarized. Finally, the problem of the role of endotoxin in the pathogenesis of the fever accompanying infections produced by Gram-negative bacteria will be discussed.

It is appropriate at this point to acknowledge the fact that fever is

[*] Presented at a Symposium on *Endotoxins and Gram-Negative Infections*, held by the Section on Medicine of The New York Academy of Medicine, March 25, 1961.

but one of the many striking biological alterations that can be induced by the administration of bacterial endotoxins. These include polymorphonuclear leukopenia followed by leukocytosis, enhanced reactivity to epinephrine and related substances, hemodynamic changes, increased or decreased resistance to infection by bacteria and viruses, destruction of tissue as exemplified by the Shwartzman reaction, potentiation of antibody response, depletion of liver glycogen, shock and vascular collapse, and, in sufficiently large dosage, death. [1-3] It is not known at present whether these effects depend upon a single basic action of endotoxin or whether several separate mechanisms are involved. Many of these effects of endotoxin are discussed in the other papers of this symposium.

The endotoxins of Gram-negative bacteria are lipopolysaccharides and, as far as is known, they bear no relationship to pathogenicity or virulence of the strain or species from which they are derived. While differing slightly in chemical composition and in antigenic properties, endotoxins from all Gram-negative bacteria are qualitatively similar in their biologic activities. Only microgram quantities are needed to elicit a pyrogenic reaction, and of great importance is their ability to resist destruction by boiling or even autoclaving.

Characteristics of Endotoxin Fever

The intravenous injection of bacterial endotoxin in man is followed by an asymptomatic interval of 30-90 minutes which terminates with abrupt onset of fever, often with a shaking chill. The fever reaches a peak within two to three hours and subsides gradually by the seventh or eighth hour. In the dog and rabbit, the "latent period" after intravenous injection is distinctly present but tends to be somewhat shorter than that in man. The febrile response lasts six to eight hours and, except with very small doses, the fever curve in these animals is *biphasic*, reaching an initial peak at about two hours, remitting slightly, then rising again and subsiding slowly to the baseline. This *biphasic* response is highly characteristic of endotoxin fever in the rabbit and the dog. Occasionally, one sees a biphasic response in man, usually in instances of unusual susceptibility to the pyrogen (as in Laennec's cirrhosis) or accidental overdosage.

Because of the latent period between direct introduction of endotoxin into the circulation and abrupt onset of fever, it was suggested

some years ago that endotoxin's effect upon the nervous centers regulating body temperature might be an indirect one, perhaps mediated by some product released from cells injured by the injected toxin.[4]

In man and animals, daily injection of endotoxin leads to the development, within five to seven days, of a state of relative nonreactivity to the fever-producing effect of the bacterial pyrogen.[4] This *tolerance* is accompanied by an increased ability of the host to clear injected toxin from the blood, and the toxin that is removed is sequestered in liver and spleen.[5, 6] This has given rise to the view that the mechanism of tolerance is an "activation" of the cells of the reticuloendothelial system (RES).

Tolerance is *never complete*, a minimal febrile response continues to appear. Tolerance is *transient* and will disappear if daily injections are stopped for two to three weeks. It is *abolished immediately by injection of colloidal substances* such as Thorotrast, which gives additional support to the idea that altered function of the RES is involved.[6]

Tolerance is *dose-dependent* and can be overcome if the amount of endotoxin injected is increased. Finally, tolerance is *not dependent on demonstrable antibody formation*, it is nonspecific in the sense that nonreactivity induced by treatment with the endotoxin of one bacterial species is effective against the endotoxins of all Gram-negative bacilli, and the tolerance is not transferable by serum with any regularity. Tolerance to daily injection of endotoxin in animals is first manifested by a disappearance of the second hump of the diphasic response, shorter duration of fever and gradual diminution in peak of the febrile response.[7] Under certain conditions, transfer of serum from tolerant rabbits to normal rabbits has appeared to modify the "second hump" in a manner suggesting that some factor in the serum might be responsible for a part of the tolerant response.[8] That humoral factors alone can account for tolerance is extremely unlikely, and others have not made the same observation when animals were challenged after transfer of tolerant serum.[4, 9]

In summary:

1. Endotoxin fever in the rabbit and the dog has its onset after a *latent period*, is typically biphasic, and subsides after several hours.

2. Daily injection of endotoxin is attended by the development of a nonspecific, incompletely transferable *tolerance* to the pyrogenic action of endotoxins.

3. Endotoxin tolerance will subside spontaneously if daily injection is stopped for about three weeks, and is abolished promptly by colloidal substances of the so-called RES-blockading type.

The Mechanism of Endotoxin Fever

Because the lag period between injection of endotoxin and onset of fever suggests so strongly that tissue injury with release of a humoral substance is an initial step in the development of fever, a careful search for an "endogenous" pyrogen was carried out. Briefly, it was found that the serum of rabbits given endotoxin contains a fever-producing substance which has properties quite unlike those of the originally injected endotoxin. This serum substance elicits a brief (three to four hour), monophasic fever after a very short lag period, it is destroyed by heating at 90°C. and it is fully active in endotoxin-tolerant rabbits.[10, 11] Furthermore, as tolerance to endotoxin develops, this material fails to appear in the circulation and daily injections of endogenous serum pyrogen elicit no tolerance in rabbits.

Polymorphonuclear leukocytes are known to contain a fever-producing substance that also differs in its properties from bacterial endotoxin.[12, 13] Indeed, the actions of leukocytic pyrogen and of the "endogenous serum pyrogen" are in all ways similar, and it is highly likely that the two represent a single substance. Despite extensive searches, the polymorphonuclear leukocyte remains the only cell with pyrogenic properties yet discovered.

All of the above findings with leukocyte extracts and with the serum of animals given endotoxin have been fully confirmed in both the rabbit and the dog.[14]

As has been mentioned briefly, the injection of endotoxin is regularly followed within minutes by a profound neutropenia with gradual "rebound" to a peripheral leukocytosis after a few hours. This sharp fall in neutrophils invariably precedes the onset of fever in endotoxin-treated animals. It is known that a large amount of injected endotoxin is immediately taken up by circulating neutrophils.[15] Also, exposure of neutrophils to endotoxin *in vitro* results in an increased release of endogenous pyrogen from these cells.[16] Therefore, what may be called the hypothesis of "indirect action" of endotoxin can be stated: The endotoxin injures host cells, including polymorphonuclear leukocytes, releasing endogenous pyrogen which, in turn, acts on cerebral centers

to elicit fever.

At this point, it should be stated that a serum pyrogen closely resembling leukocytic pyrogen has also been shown to mediate several other types of experimental fever. These include the fevers produced by pneumococcal and streptococcal infections, after injection of myxoviruses, after injection of staphylococci and their products, and after hypersensitivity reactions of the immediate and the delayed types.[17]

Examination of evidence that endogenous pyrogen is the sole mediator of the febrile response to injected endotoxin reveals possible inconsistencies that deserve study. At certain stages during the development of tolerance and in other experimental situations, the febrile response of an animal given endotoxin is poorly correlated with the amount of endogenous pyrogen demonstrable in the circulation.[11, 18] Even granting that the only available method for detecting serum pyrogen is relatively crude bioassay, the discrepancies are striking at times. It has also been shown that the intrathecal instillation of endotoxin in amounts far smaller than are required by vein will elicit an immediate, intense febrile response.[18] Tolerance does not develop to repeated injections of endotoxin by this route, the fever is not preceded by leukopenia, and endogenous pyrogen is not demonstrable in the blood of animals made febrile by this method. When large doses of endotoxin are given intravenously, the cerebrospinal fluid is found to contain significant amounts of this material within 15 to 20 minutes.[18] When endogenous serum pyrogen is infused into the carotid artery, it elicits a greater fever than that seen when the intravenous route is used, strong evidence for a direct action of this substance upon the brain;[19] endogenous serum pyrogen is also highly active when given intrathecally.[18] When endotoxin is infused by carotid artery, the febrile response is no greater than that seen when the intravenous route is used.[19] This last result, however, cannot be taken as a serious obstacle to a direct action of endotoxin on the brain for several reasons. First, the dosage and infusion times were selected deliberately to show the widest possible difference for serum pyrogen. Second, while slow infusion of serum pyrogen may well duplicate its release *in vivo*, it is known that the concentration of endotoxin in the circulation after a single injection reaches a peak within a few minutes and then falls off rapidly. The lipopolysaccharide molecule is a large one, and its penetration into the nervous system may well be slower than that of endogenous pyrogen. However, the endo-

toxin is *always* present in the circulation *before* endogenous pyrogen appears, and this "headstart" was not allowed for in the infusion experiments either.

The final settlement of the question of "indirect" or combined "indirect" and "direct" action of endotoxin will come perhaps from additional studies utilizing more refined neuropharmacologic techniques. Careful experiments with neutropenic animals (given nitrogen mustard or exposed to ionizing radiation) are also greatly needed and are under way in several laboratories.

For the present, however, it can be concluded that the traditional endotoxin fever model is probably a great deal more complicated than has heretofore been suspected.

The Role of Endotoxin in the Fever of Infection

While it is logical to suppose that the endotoxin fraction plays an important role in the genesis of the fever and of other manifestations accompanying active infection by Gram-negative bacteria, objective evidence to support this idea is surprisingly scanty.

Because of the striking development of tolerance to the pyrogenic action of repeated injections of endotoxin in man or animals, it has been assumed that patients convalescent from febrile diseases produced by Gram-negative bacteria might show resistance to the pyrogenic action of a test dose of endotoxin. Furthermore, because endotoxin tolerance subsides rapidly when exposure is stopped, the tolerance of the convalescent might also be expected to wane with passing weeks.

A study of the reactivity of rabbits after infection by colon bacilli failed to demonstrate tolerance to homologous or heterologous endotoxin.[20] However, the infection produced in these animals was not accompanied regularly by bacteremia, a point which may be of significance.

Occasional testing of man during recovery from a variety of bacterial infections led to no firm conclusions.[21] However, recovery from malaria carries with it a significant resistance to the pyrogenic action of typhoid endotoxin, a finding that may be explicable on the basis of enhanced activity of the RES in this parasitic disease.

Another study utilizing patients who had become afebrile after typhoid or paratyphoid fever showed conclusively that most subjects were refractory to induced endotoxin fever.[22] Until recently, this was

the best evidence available for a role of endotoxin in infection. Interestingly enough, no tolerance was demonstrable in patients recovering from bacillary dysentery, a disease which elicits high fever but in which the infecting organism is limited to the lumen of the intestine and almost never invades the circulation.

A study of the response to endotoxin of patients convalescing from brucellosis showed them to be abnormally susceptible to the pyrogenic action of this material.[23] This is difficult to explain, but it may well raise the question of hypersensitivity (in the immunologic sense) as a basic mechanism in the action of endotoxins.

Finally, included in this symposium is a description of more recent and detailed studies of possible action of endotoxin in the pathogenesis of typhoid fever.[24] The contemplated extension of these investigations can be expected to provide a good foundation for understanding endotoxin's part in the fever of infection.

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THE INFLUENCE OF ENDOTOXIN ON RESISTANCE TO INFECTION*

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Endotoxins of Gram-negative bacteria can either raise or lower resistance to infection. This variable effect can be partly explained by the dual nature of endotoxin, a substance that is both antigenic and toxic. Toxicity takes the form of abrupt disturbances in leukocytes, body temperature, circulatory dynamics, intestinal motility, carbohydrate metabolism, serum proteins, and endocrine secretion. During the course of these profound abnormalities, it is not surprising that resistance to infection may fall sharply. The antigenic activity of endotoxins, on the other hand, may elevate resistance to infection by stimulating specific immunity to the homologous bacteria. The Gram-negative endotoxins have been designated "O" antigens and their specific immunologic properties provide the only means of distinguishing between endotoxins of different bacterial species. In contrast to these antigenic differences, there is striking uniformity in toxic manifestations of endotoxins from all Gram-negative bacteria.

While these generalizations do not account for all changes in resistance that follow the administration of endotoxins, they serve as a basis for examining the problem at greater length. The following analysis will consider in turn the unfavorable and favorable influence of endotoxin on infection.

LOWERED RESISTANCE

LOCAL INFECTION

The effects of endotoxin on resistance to local infection have been more dramatic and convincing than on resistance to lethal systemic infections. The enhancement of local infections by endotoxin was fully

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TABLE I—ENHANCEMENT OF BACTERIAL SKIN LESIONS WITH CRUDE E. COLI ENDOTOXIN IN RABBITS

Rabbit Number	*Inoculum*	*Size of Lesion (mm²)* 15 Hours	24 Hours
1	2 mg. Endotoxin	0	0
	10^7 S. aureus	0	0
	2 mg. Endotoxin plus 10^7 S. aureus	227 I 36 N	285 N
2	2 mg. Endotoxin	0	0
	10 mg. Endotoxin	0	100 I
	10^7 S. aureus	0	0
	10^7 Cl. perfringens	0	0
	2 mg Endotoxin plus 10^7 S. aureus	417 I 28	810 I 361 N
	2 mg Endotoxin plus 10^7 Cl. perfringens	0	94 I
	10 mg. Endotoxin plus 10^7 Cl. perfringens	36 N	400 N

I = Induration
N = Necrosis

Crude *E. coli* endotoxin consisted of dried bacterial bodies washed from tryptose agar after growth at 37°C. for 72 hours. The *E. coli* were washed with 95% ethyl alcohol twice and acetone ether once. They were brought to dry weight and stored in a dessicator. The LD_{50} in mice was .312 mg. Rabbits were inoculated intradermally on the back with the various materials suspended in a final volume of 1.0 ml. sterile saline. Staphylococcus cultures were grown on 10% rabbit blood agar and clostridia in Brewer's liquid thioglycollate medium for 24 hours and resuspended in saline.

described by Miles and Niven.[1] They found that skin infections by *Staphylococcus aureus*, *Clostridium perfringens*, and *Pseudomonas aeruginosa* were intensified in guinea pigs receiving intraperitoneally large sublethal doses of endotoxins within two to three hours of the intradermal injection of bacteria, but not later. Intensification of local infection was reproduced by intraperitoneal injection of endotoxins from various Gram-negative bacteria. Upon analyzing the mechanism of this enhancement, Miles and Niven learned that the two most critical factors were severe hypotension lasting for at least one hour, and an absence of tissue leukocytes. The deficiency in leukocytes in the infected area was probably due to a reduction in the flow and pressure of blood in skin capillaries so that the supply of blood-borne leukocytes was diminished. The primary leukopenic effect of endotoxin was not essential because tissue leukopenia and enhanced skin infections were

also produced by a variety of shocking agents, including clostridial exotoxin, staphylococcal exotoxin, magnesum adenose triphosphate and intraperitoneal hypertonic glucose.

Evans, Miles, and Niven[2] also reported that simultaneously injected epinephrine temporarily diminished the supply of blood and phagocytes to the infected lesions, and suggested that the associated enhancement of infection was a consequence of this local inhibition of diapedesis. The possibility that endotoxin itself might act locally at the site of staphylococcus infection was investigated in our laboratory soon after the report of Miles and Niven. We found no effect when purified *E. coli* endotoxin was injected simultaneously with viable bacteria, but crude preparations of dried bacterial bodies of *E. coli*, containing approximately 20 per cent endotoxin, markedly intensified staphylococcal and clostridial skin lesions in rabbits without producing hypotension or shock. As shown in Table I, the crude endotoxin preparations induced severe infected skin lesions with bacterial inocula that produced no lesions alone. Although no explanation has been found for the different behavior of purified and crude endotoxins in local infections, the clinical implication is inescapable that clostridial and staphylococcal infections could be seriously aggravated by contamination with relatively nonpathogenic Gram-negative bacteria.

SYSTEMIC INFECTION

The survival of infected animals can be shortened by administering endotoxins either before or after the infection is introduced. Large doses of endotoxin, in the range of the LD_{50}, are usually required for both effects. When prior injection of endotoxin lowers resistance of mice to systemic infection, deaths occur shortly after challenge with viable bacteria. The fast-death rate, as well as the high toxicity of the bacteria (*S. aureus*[3] and *E. coli*[4]), indicate that the animals die of overwhelming toxemia. This likelihood is supported by the fact that large doses of endotoxin and bacteria must be given almost simultaneously to induce faster killing. The observation by Gale and Sandoval,[5] that 5 million cells of *Candida albicans* kill mice faster if they receive *E. coli* endotoxin 30 minutes after infection, is probably another example of a combined effect of two toxic agents administered in close succession.

The mechanism of accelerated deaths after injection of endotoxin

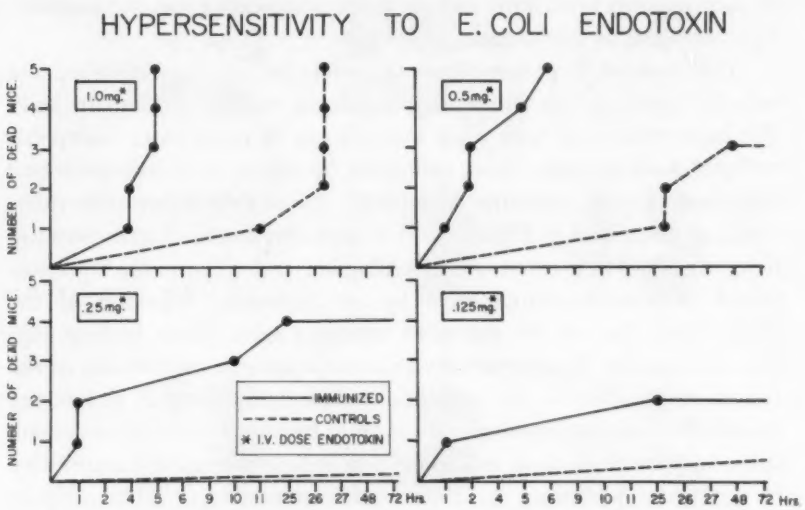

Figure 1—Immunized white Swiss mice (26-31 gm.) received intraperitoneally .005 mg. *E. coli* endotoxin on day 1, .01 mg. on day 2, and .02 mg. on days 6, 8, 10, and 13 for a total of 6 injections. Controls received intraperitoneal saline by the same schedule. Four days after the last injection all mice were challenged with graded doses of endotoxin as shown. The LD_{50} in the controls was .481 mg. and in the immunized hypersensitive mice it was .194 mg. ($P = < .01$). The challenge doses of endotoxin were given intravenously.

in animals with established infections is less obvious. Brucellosis and salmonellosis in mice, tuberculosis and Q-fever in guinea pigs, and *E. coli* peritonitis in rabbits lower resistance to the lethal effect of endotoxin for periods up to three months after the onset of infection.[6-11] Boivin and Mesrobeanu[7] have suggested that infected animals owe their increased susceptibility to an explosive reactivation of the infection by endotoxin. Their view is not consistent, however, with the finding of Miles, that dermal infections older than three hours could not be intensified by endotoxin; with the meticulous studies of Abernathy, Bradley, and Spink[6] who found no increase in the number of brucellae cultured from the spleens after injection of endotoxin; or with the failure of Pirsch, Mika, and Van Der Maaten[10] to demonstrate an increase of *C. burnetti* in the blood or spleens of infected guinea pigs after treatment with brucella endotoxin. The rapid deaths of the infected mice are better explained by hypersensitivity to the endotoxin. Both dermal and systemic hypersensitivity to endotoxin has been found

in patients with brucellosis and probably represents a specific immunologic response to the somatic antigen.[12]

This type of hypersensitivity to endotoxin can be established not only by infection but also by immunization with the endotoxin itself. We have found that both mice and rats can be made more susceptible to lethal doses of homologous endotoxin by one or more intraperitoneal injections of small quantities of either *E. coli* or *Proteus mirabilis* endotoxin, as illustrated in Figure 1. We have also found that hypersensitivity to homologous, but not heterologous, endotoxin is readily transferred to non-immunized mice by intraperitoneal injection of the whole blood, but not the serum, of sensitized mice. These findings suggest that specific hypersensitivity to somatic antigen may be one of the factors responsible for the increased lethality of endotoxin in infected animals. In addition, a non-specific form of hypersensitivity to endotoxin seems to operate in those infections that induce increased susceptibility to endotoxins possessing no demonstrable immunologic relationship to the infectious agent. Lowered resistance to endotoxin is also seen in animals after treatment with thorotrast[13] or pertussis vaccine.[14] The action of thorotrast may depend on its damage to the reticuloendothelial system, while the pertussis effect has been attributed to the release of lethal quantities of histamine and serotonin upon challenge with endotoxin. Neither of these mechanisms has been seriously investigated in infected animals and the basis for their non-specific hypersensitivity to endotoxin remains obscure.

Increased Resistance

LOCAL INFECTION

Pyelonephritis and cholecystitis are the most common localized infections due to Gram-negative bacteria in man. The recent development of a well standardized experimental model has permitted us to study the effect of endotoxin on the course of hematogenous pyelonephritis.[15] When adult rats are immunized with three intraperitoneal injections of an endotoxin extracted from the infecting bacteria, the severity of hematogenous pyelonephritis due to *E. coli* or *P. mirabilis* is greatly reduced. Figure 2 records the marked reduction in severity of pyelonephritis in rats inoculated intracardially with 10^8 *E. coli* 0:113 one week after the third weekly immunizing dose of .01 mg. *E. coli* 0:113 endotoxin. The animals had been subjected to light renal

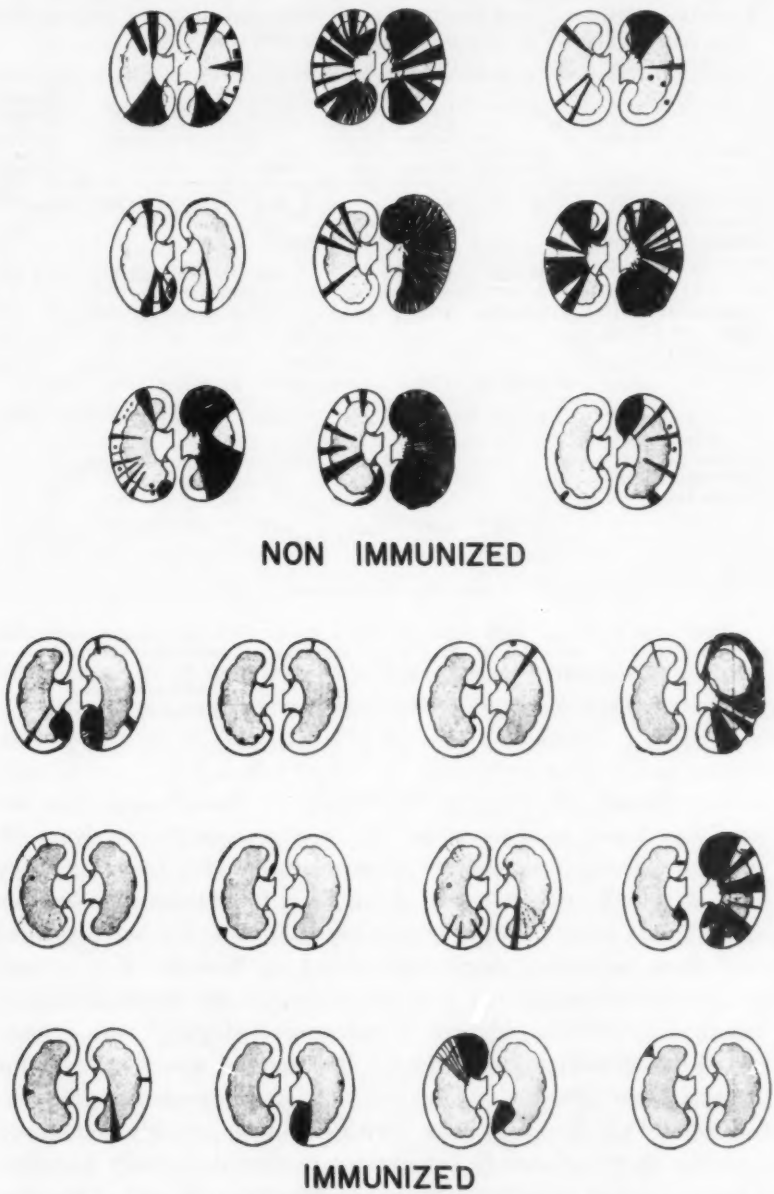

Figure 2—Diagrammatic portrayal of gross pyelonephritis produced by *E. coli*. Immunized rats received 3 injections at weekly intervals of .01 mg. *E. coli* endotoxin. Gross lesions are indicated by shaded areas.

TABLE II—IMMUNIZATION AGAINST PYELONEPHRITIS WITH ENDOTOXIN OR DETOXIFIED ENDOTOXIN

Immunizing Agent	*Bacteria Inoculated*	*Death Rate** Deaths Total Animals*	*Average Weight Change per 350 gram Rat (grams)*	*Severity of Pyelonephritis (average grade per kidney)*	*% Kidneys with Lesions Grade 1 or Worse*	*Average Number Viable Bacteria per gm. Kidney*
E. coli Endotoxin (.01 mg. I.P. weekly x 3)	E. coli	0/12	*	1.31	54% (13/24)	4.1×10^3
0	E. coli	4/13	*	3.28	100% (18/18)	8.1×10^4
Proteus mirabilis Endotoxin (.01 mg. I.P. weekly x 3)	P. mirabilis	0/12	+8.8	1.55	83⅓% (20/24)	4.85×10^4
0	P. mirabilis	2/12	−13.8	2.47	85% (17/20)	4×10^4
$LiAlH_4$-treated Detoxified E. coli Somatic Antigen (.001 mg. and .019 mg. ten days later)	E. coli	0/20	+5	1.05	50% (20/40)	1.0×10^3
0	E. coli	3/20	−23	2.15	91% (31/34)	24×10^3

* No determination.
** Total deaths immunized = 0/44; total deaths non-immunized = 9/45.

Rats were challenged with viable *E. coli* 3 weeks after beginning immunization, or with viable *P. mirabilis* 25 days after the start of immunization. Lesions in kidneys were graded according to the scale in Figure 5, where shaded areas represent the extent of gross pyelonephritis. Average severity $= \frac{\text{sum of individual grades}}{\text{number of kidneys}}$

massage through the intact abdominal wall for five minutes after bacterial inoculation in accordance with the technique previously developed for eliciting pyelonephritis without urinary obstruction, and they were sacrificed after one week of infection. In addition to protection against gross renal damage, immunization with endotoxin prevented death from bacteremic shock and reduced significantly ($P = < .01$) the number of bacteria per gram of kidney. Similar results have been described by Sanford, Hunter, Windom, and Hogan,[16] who demonstrated that specific antibody to the "O" antigen was responsible for the protection afforded by immunization against pyelonephritis with endotoxin. In a key experiment they found that passively transferred antibody against *E coli* O:111 antigen conferred virtually complete resistance against pyelonephritis due to homotypic *E. coli*. They also reported that o:111 antibody did not offer protection against renal

TABLE III—EFFECT OF IMMUNIZATION WITH E. COLI ENDOTOXIN ON LOCALIZATION AND GROWTH OF E. COLI IN SPLEENS REMOVED 5 MINUTES AFTER INTRACARDIAC INOCULATION

Immunized				*Non-Immunized*			
		Viable E. coli per Gram of Spleen				*Viable E. coli per Gram of Spleen*	
Rat No.	*Wt. of Spleen Grams*	*Upon Removal from Rat*	*After 3 Hrs. at 37° C.*	*Rat No.*	*Wt. of Spleen Grams*	*Upon Removal from Rat*	*After 3 Hrs. at 37° C.*
1	1.1879	17,802,000	87,400,000	3	1.3497	16,995,000	110,826,000
2	1.8100	7,508,000	48,185,000	4	1.0689	23,436,000	78,210,000
5	1.3472	6,976,000	44,388,000	12	2.1361	22,277,000	114,654,000
6	1.2588	15,735,000	25,912,000	13	2.4576	15,333,000	45,582,000
14	1.1402	5,814,000	88,560,000	16	1.1413	25,240,000	124,260,000
15	1.6177	14,300,000	138,240,000	23	1.5084	16,993,000	96,600,000
	Average	11,356,000	72,131,000		Average	20,212,000	95,022,000

Immunized rats received a total of 3 intraperitoneal injections of *E. coli* endotoxin prepared from bacteria grown in synthetic medium.[34] A dose of 0.1 mg. endotoxin was given at each injection and the interval between injections was 1 week. Thirteen days after the last immunizing dose, rats were inoculated intracardially with 10^6 *E. coli* (18 hour broth culture) and the kidneys massaged for 5 minutes through the intact abdominal wall. Five minutes after bacterial inoculation each spleen was removed, weighed, and cut in half. Bacterial counts were performed immediately on one-half after homogenization. The other half was placed at 37°C. in moist chambers for 3 hours and then homogenized for bacterial counts.

infection with Klebsiella type C or heterotypic strains of *E. coli*. We have also established the importance of the somatic antigen in protection against pyelonephritis by immunizing rats with a preparation of endotoxin treated with $LiAlH_4$ by Dr. Hans Noll.[17] The product of such treatment was non-toxic but fully antigenic and not only induced pyrogen tolerance, formation of humoral antibodies, and lethal tolerance, but also provided marked protection for rats inoculated intracardially with the homologous *E. coli* (Table II). Both the severity and incidence of pyelonephritis was much less in animals immunized with minute amounts of $LiAlH_4$-treated endotoxin than in those not immunized. Although the average number of viable bacteria per kidney was less in the immunized group, it was observed that immunization greatly reduced severity of pyelonephritis in individual kidneys which contained the same number of viable bacilli as non-immunized controls. The discrepancy between persistence of viable bacteria and reduction of renal damage was even more obvious in animals immunized with Proteus endotoxin. Such immunization cut the severity of Proteus pyelonephritis by nearly one-half but did not lower the bacterial count

TABLE IV—BLOOD CLEARANCE OF E. COLI IN RATS IMMUNIZED WITH HOMOLOGOUS ENDOTOXIN

Immunized				*Non-Immunized*			
Time After Intracardiac Inoculation	*Rat No.*	*Viable Bacteria per ml Blood*		*Time After Intracardiac Inoculation*	*Rat No.*	*Viable Bacteria per ml Blood*	
		Individual	*Average*			*Individual*	*Average*
	25	2,307,000			56	1,084,500	
5 min.	34	1,101,000		5 min.	45	1,694,000	
	4	56,000			35	1,362,000	
	16	975,000			145	669,000	
			1,109,800				1,202,400
	13	1,477			123	43,500	
1 Hour	23	475		1 Hour	156	2,190	
	6	2,157			234	103,770	
			1,370				49,820
	5	960			36	1,020	
2 Hours	1	330		2 Hours	125	3,527	
	14	1,810			136	1,487	
	15	11,270			46	22,800	
			3,590				7,210
	12	540			134	700	
24 Hours	24	250		24 Hours	135	790	
	3	155			126	6,030	
	26	165			124	180	
			280				1,925

The conditions of the experiment were the same as those in Table III, except that instead of splenectomy, rats were subjected to cardiac puncture to obtain blood for plate counts at 5 min., 1 hour, 2 hours and 24 hours after inoculation of *E. coli*.

in the kidneys (Table II). These results suggest that immunization may protect the kidney from the direct action of endotoxin despite unrestricted bacterial growth. The protection against the lethal toxicity of acute bacteremia, as summarized in Table II, also favors the idea that immunization has antitoxic value in infection by Gram-negative bacteria.

The effect of immunization with endotoxin on antibacterial defense in pyelonephritis was also investigated by comparing splenic localization of bacteria, blood clearance of bacteria, and bactericidal activity of tissues and serum in immunized and control animals. Rats were sacrificed 5 minutes after intracardiac inoculation with *E. coli* when maximum renal localization of bacteria occurs,[15] and the spleens removed and weighed. Half the spleen was homogenized immediately for bacterial counts and the remainder incubated at 37°C. for three hours when quantitative cultures were also performed. As shown in Table III,

TABLE V—EFFECT OF IMMUNIZATION WITH E. COLI ENDOTOXIN ON LOCALIZATION AND GROWTH OF E. COLI IN KIDNEYS REMOVED 5 MINUTES AFTER INTRACARDIAC INOCULATION

Immunized				*Non-Immunized*			
		Viable E. coli per Gram of Kidney				*Viable E. coli per Gram of Kidney*	
Rat No.	*Wt. of Kidneys Grams*	*Upon Removal From Rat*	*After 3 Hrs. at 37° C.*	*Rat No.*	*Wt. of Kidneys Grams*	*Upon Removal From Rat*	*After 3 Hrs. at 37° C.*
2 R	1.4585	1,275,000	44,403,000	4 R	1.4587	3,612,000	39,285,000
L	1.3882	1,308,000	40,719,000	L	1.4519	3,595,000	46,599,000
5 R	1.3540	2,016,000	11,226,000	12 R	1.4763	2,982,000	15,522,000
L	1.3063	1,570,000	25,080,000	L	1.4939	4,296,000	47,265,000
6 R	1.6729	1,798,000	117,000,000	13 R	1.4198	2,898,000	14,014,000
L	1.3712	832,000	27,400,000	L	1.3442	2,099,000	8,436,000
14 R	1.1182	1,533,000	60,090,000	16 R	1.2016	5,038,000	63,360,000
L	1.2379	1,684,000	168,400,000	L	1.1794	6,256,000	93,624,000
15 R	1.4386	890,000	23,326,000	23 R	1.3598	4,662,000	38,069,000
L	1.3071	878,000	51,800,000	L	1.3374	3,448,000	100,815,000
	Average	1,378,000	56,944,000		Average	3,889,000	46,699,000

The details of the experiment are given in Table III.

immunization did not increase the uptake of bacteria into the spleen during the critical first five minutes of maximal renal localization nor did spleens of immunized rats possess enhanced bactericidal activity as measured *in vitro*. Instead, there seemed to be a slight reduction in splenic uptake of *E. coli* by immunized rats. Clearance of *E. coli* from the blood during the first five minutes was also not increased by immunization, but afterwards there was a suggestion that bacteremia was slightly but irregularly reduced in immunized rats (Table IV). Localization of *E. coli* at five minutes in massaged kidneys also appeared slightly but consistently reduced in immunized rats as measured by plate counts of homogenized kidneys (Table V). Since equal numbers of *E. coli* were delivered in the blood of both groups of rats at that time, it was suspected that bacterial agglutinins, constantly present in immunized animals only, clumped the organisms in the kidney and spleen and thereby reduced the plate counts. This possibility was strengthened when it was found that upon incubation of the kidneys at 37°C. for three hours *in vitro*, the bacterial counts in the kidneys of immunized and non-immunized rats rose to approximately equal levels. This experiment, summarized in Table V, further illustrates that

TABLE VI—EFFECT OF IMMUNIZATION WITH E. COLI ENDOTOXIN ON BACTERICIDAL POWER OF RAT SERUM AGAINST E. COLI

Immunized					*Non-Immunized*				
		Viable Bacteria per 0.1 ml. Serum					*Viable Bacteria per 0.1 ml. Serum*		
Rat No.	*Hemagglut. Titer (Reciprocal)*	*0 Hrs.*	*1 Hr.*	*24 Hrs.*	*Rat No.*	*Hemagglut. Titer*	*0 Hrs.*	*1 Hr.*	*24 Hrs.*
1	128	2355	0	0	1	2	2955	2410	0
2	64	2025	1950	0	2	2	2745	3135	0
3	128	1845	2205	0	3	0	2640	2850	0
4	128	2220	1920	0	4	2	3000	3285	0
5	64	2265	2385	0	5	2	2820	3750	0
6	64	1980	2100	1	6	2	2625	2520	0
12	256	2355	3585	0	12	0	2625	3010	0
13	128	1620	2235	0	13	2	2925	3000	0
14	128	3405	2205	3	14	2	3060	3075	3
15	256	1935	2880	0	15	4	2745	2850	1

Immunized rats received a total of 3 intraperitoneal injections of *E. coli* endotoxin 0:113. A dose of .01 mg. endotoxin was given at each injection and the interval between injections was 1 week. Blood for bactericidal test was drawn 12 days after the last dose of endotoxin and the test was performed then on fresh serum inoculated with an 18-hour tryptose broth culture of the homologous strain of *E. coli* 0:113. Inoculated sera were incubated at 37°C. for 24 hours and 0.1 ml. samples removed at specified intervals for plate counts in tryptose agar. The hemagglutination test for antibody to *E. coli* endotoxin was performed by the technique of Neter.[22]

immunization with endotoxin did not confer bactericidal activity on the renal tissues.

Immunization also failed to improve the bactericidal action of serum. Sera of non-immunized rats possessed strong bactericidal power against the strain of *E. coli* used for induction of pyelonephritis and usually sterilized inocula in the range 3×10^4 bacteria per ml. within 24 hours at 37°C. Similar inocula of Proteus organisms, however, were not sterilized by sera of non-immunized rats but moderate killing was often obtained. Rat sera were equally bactericidal for *E. coli* before and after immunization with *E. coli* endotoxin (Table VI). After immunization with Proteus endotoxin, on the other hand, rat sera lost their weak bactericidal power for Proteus, as shown in Table VII. In spite of this unfavorable change *in vitro*, the immunized rats suffered much less severe pyelonephritis than controls upon inoculation with Proteus immediately after the blood was obtained for bactericidal testing.

Although bactericidal power was not improved, immunization stimulated high titers of hemagglutinating antibody to the "O" antigen of

TABLE VII—EFFECT OF IMMUNIZATION WITH PROTEUS MIRABILIS ENDOTOXIN ON BACTERICIDAL POWER OF RAT SERUM AGAINST *P. MIRABILIS*

Immunized					*Non-Immunized*				
Rat No.	*Hemagglut. Titer (Reciprocal)*	*Viable Bacteria per 0.1 ml. Serum*			*Rat No.*	*Hemagglut. Titer (Reciprocal)*	*Viable Bacteria per 0.1 ml. Serum*		
		0 Hrs.	*1 Hr.*	*24 Hrs.*			*0 Hrs.*	*1 Hr.*	*24 Hrs.*
1	512	6410	5426	10^7	56	2	2400	1095	382
2	128	2910	2265	10^7	124	2	2160	1320	10^7
5	1024	2625	2385	10^7	126	0	1860	1200	274
6		2400	2250	10^7	134	0	2250	1365	10^7
11	512	2550	2640	10^7	136	0	2565	990	74
13	512	2670	2625	10^7	145	2	2670	1275	10^7
14	8192	2430	2280	10^7	146	2	2685	1230	538
15	256	3070	3705	10^7	156	2	2445	1155	1515

Immunized rats received a total of 3 intraperitoneal injections of *Proteus mirabilis* endotoxin prepared by bacteria grown in synthetic medium.[34] A dose of .01 mg. endotoxin was given at each injection and the interval between injections was 1 week. Blood for bactericidal test was drawn 12 days after the last dose of endotoxin and the rats were challenged immediately with viable *P. mirabilis* and the results are given in Table II. The bactericidal test was performed on fresh serum inoculated with an 18-hour tryptose broth culture of *P. mirabilis*. Inoculated sera were incubated at 37°C. for 24 hours and 0.1 ml. samples removed at specified intervals for plate counts in tryptose agar. The hemagglutination test for antibody to *P. mirabilis* endotoxin was performed by the technique of Neter.[35]

TABLE VIII—HEPATIC LOCALIZATION OF E. COLI ENDOTOXIN UPON RECOVERY FROM PYELONEPHRITIS IN RATS

Time of sacrifice (minutes)	*% Total injected radioactivity in liver: Mean Values*	
	Pyelonephritic	*Normal Control*
0-1	10.22	Not determined
15	20.5	8.4
30	32.3	16.2
60	39.8	14.9

the respective bacteria. Another rise in hemagglutinating antibody occurred one week after infection in both immunized rats and controls. At the end of six weeks the titers had fallen but clearance studies showed that recovery from *E. coli* pyelonephritis was accompanied

PLASMA CLEARANCE RATES OF ENDOTOXIN IN NORMAL RATS AND IN RATS RECOVERING FROM E. COLI PYELONEPHRITIS

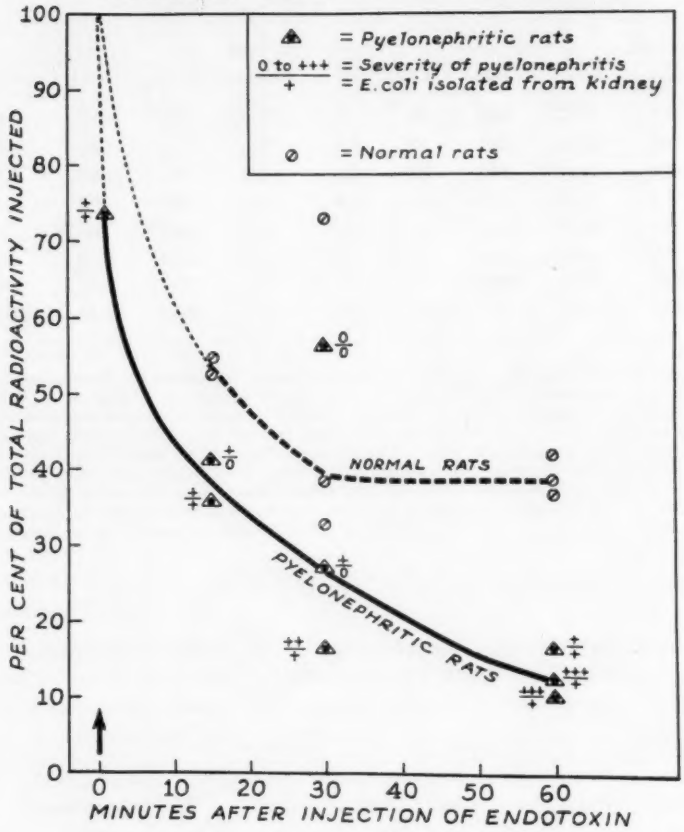

Figure 3—Six weeks after infection, each pyelonephritic rat and normal control received intravenously 5.0 mg. of *E. coli* endotoxin labelled with radioactive sodium chromate (total counts injected per rat = 39,000 counts per second) and were sacrificed at the time intervals shown in the figure. The endotoxin was prepared and labelled by techniques described elsewhere.[36]

by increased removal of radioactive *E. coli* endotoxin from the blood into the livers. A comparison of the clearance rates given in Figure 3 discloses that one hour after the intravenous injection of 5.0 mg., the concentration in the plasma had fallen almost to 10 per cent (mean

= 12.9) in the pyelonephritic rats while in normals it remained above 36 per cent (mean = 38.9). The faster removal of endotoxin by pyelonephritic rats was accompanied by a much greater hepatic uptake than normal, as shown in Table VIII. The specificity of this phenomenon is brought out by the fact that in rats with Proteus pyelonephritis the distribution curves of *E. coli* endotoxin were the same as normal. Upon recovery from *E. coli* pyelonephritis, the kidneys of rats exhibiting enhanced clearance of *E. coli* endotoxin into the liver were resistant to gross pyelonephritis upon re-inoculation of *E. coli* and renal massage.

It would appear from these studies of pyelonephritis that resistance to local infection by Gram-negative bacteria depends more on the development of specific resistance to endotoxin than to an increased capacity for bacterial destruction. Further support for this concept is found in the work of Freter[18] who examined the nature of resistance to experimental cholera. He found that passive oral immunization with O antiserum-protected guinea pigs against fatal enteric cholera without reducing the number of vibrios in the intestine of infected animals.

In addition to specific protection against local Gram-negative bacterial infection, non-specific protection against viral encephalitis has been produced with endotoxin by Wagner, Snyder, Hook, and Luttrell.[19] A single dose of purified endotoxin from *Salmonella abortus equi*, administered by various routes 24 hours before challenge, often enhanced the resistance of mice to intracerebral infection with the viruses of eastern equine encephalomyelitis (EEE) and encephalomyocarditis (EMC). The resistance was characterized by moderate reduction in death rate and slight prolongation of survival time. Endotoxin also produced moderate inhibition of multiplication of EEE virus in the brain, but not in tissue culture. This ineffectiveness in tissue culture distinguished the action of bacterial endotoxin from that of the viral interference phenomenon. The mechanism of this non-specific protection is entirely obscure.

SYSTEMIC INFECTION

Various endotoxins can induce in mice a rise in resistance to rapidly lethal infections by Gram-negative bacteria. Two patterns of resistance to such infections have been described. In one pattern, increased resistance develops within a few hours after the injection of endotoxins intraperitoneally or intravenously in mice and lasts for a few days only.[20-22]

The other pattern is that of classical acquired immunity, developing after a week, and persisting for weeks or months.

Early resistance. The first pattern of resistance was thoroughly explored by Landy and Pillemer,[22] who found that among 18 endotoxins examined, all induced rapid resistance to *S. typhosa* infection of mice, regardless of the method of extraction or the bacterial species from which they were derived. They also found that 1 μg. *S. typhosa* endotoxin rapidly stimulated highly effective resistance against heterologous lethal infections due to *E. coli*, *P. vulgaris*, *P. aeruginosa*, and *Klebsiella pneumoniae*. They were unable to induce with endotoxin similar resistance in mice to infection by *S. aureus*, *Streptococcus pyogenes*, or *Diplococcus pneumoniae* and concluded that the increased resistance to infection evoked in mice by endotoxins was effective only against infections produced by endotoxin-bearing organisms.

Hook and Wagner[23] also failed to alter the susceptibility of mice to Gram-positive bacteria by prior injection of endotoxin; and emphasized the exacting conditions required for inducing with endotoxin the rapid form of resistance to typhoid infection. The most effective protection was obtained when pretreatment with endotoxin and challenge with typhoid bacilli were given by the same route. For this reason they suggested that local alterations of tissue susceptibility occur at the site of injection of endotoxin.

In analyzing the nature of the rapidly-induced protection, Landy and Pillemer[22] observed that endotoxin stimulated a rise in the level of serum properdin, a substance with bactericidal properties for Gram-negative bacilli but not Gram-positives. Properdin increased at a time when the numbers of viable intra-peritoneal typhoid bacilli fell somewhat, but the bacterial reduction was slight compared to that observed after immunization with Vi antigen. This persistence of Gram-negative bacilli at high levels in protected animals suggested that tolerance to their endotoxin may contribute to their survival. Creech, Hankwitz, and Wharton[24] have demonstrated that sublethal injections of endotoxin protect mice against a subsequent lethal challenge. This protection is observed within 16 hours, is effective against both homologous and heterologous endotoxins, and persists for weeks or months.[22, 25] In addition to tolerance to lethality, endotoxin can also induce tolerance to fever, leukopenia, the Shwartzman reaction, and tumor necrosis. The relationship between various forms of tolerance is not clear, nor are the

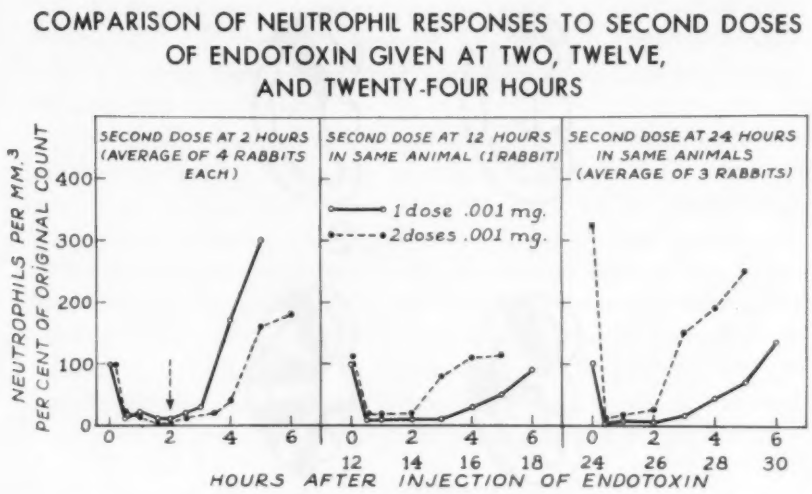
COMPARISON OF NEUTROPHIL RESPONSES TO SECOND DOSES OF ENDOTOXIN GIVEN AT TWO, TWELVE, AND TWENTY-FOUR HOURS
SECOND DOSE AT 2 HOURS (AVERAGE OF 4 RABBITS EACH)
SECOND DOSE AT 12 HOURS IN SAME ANIMAL (1 RABBIT)
SECOND DOSE AT 24 HOURS IN SAME ANIMALS (AVERAGE OF 3 RABBITS)
1 dose .001 mg.
2 doses .001 mg.
NEUTROPHILS PER MM.3 PER CENT OF ORIGINAL COUNT
400
300
200
100
0
0 2 4 6
12 14 16 18
24 26 28 30
HOURS AFTER INJECTION OF ENDOTOXIN

Figure 4—Each albino rabbit (3-4 kg.) received two successive intravenous injections of .001 mg. *E. coli* endotoxin at the intervals indicated. Blood counts were performed on the central vein of the ear.

mechanisms well understood. At certain stages of tolerance in mice and rabbits, the reticuloendothelial system can clear lethal doses of radioactive endotoxin from the blood more rapidly than in non-tolerant animals;[26] but during the first 12 hours of tolerance, clearance of lethal doses is not enhanced.[27]

Perhaps the earliest demonstrable antibacterial response to endotoxin is the marked leukocytosis that develops four hours after the injection of as little as 1 μg. endotoxin and persists for more than 24 hours. By 12 hours leukopenic tolerance has also occurred, so that a second challenge with endotoxin elicits a much shorter period of leukopenia than the first (Figure 4). It has also been reported by Cohn and Morse[28] that endotoxin enhances phagocytosis, and by Strauss and Stetson[29] that endotoxin markedly stimulates leukocytic respiration. These effects of endotoxin on leukocytes seem to represent an overall improvement in total phagocytic response by the host and might contribute greatly to the early resistance to infection.

Aside from the possible influence of properdin, reticulo-endothelial function, and leukocytic activity, other factors such as natural antibody and "acute phase" substances could contribute to the early

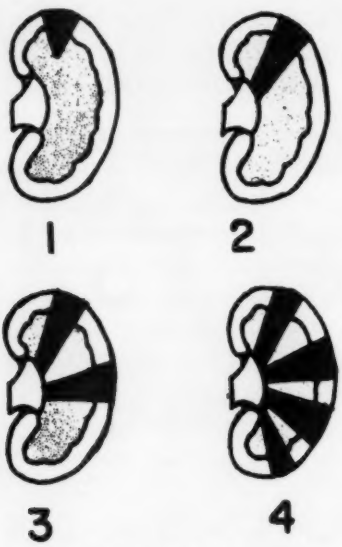

Figure 5—Grade of severity.

resistance against lethal infections. It is most probable that not one, but a combination of forces are jointly responsible for this early immune response.

Late resistance. The induction with endotoxin of the classical type of prolonged acquired immunity to systemic infection has not been studied as extensively as the rapidly developing transient form of resistance.

Boivin[30] was the first to demonstrate that *S. typhi* endotoxin could produce active immunity in mice against typhoid infection and that the immunizing fraction was the non-toxic polysaccharide. Landy, Johnson, Webster, and Sagin[31] have found more recently that a purified, protein-free endotoxin of *S. typhosa* is less effective than Vi antigen in stimulating active immunity in mice, but produces in rabbits highly potent antisera that provide a high degree of passive protection against *lethal* mouse typhoid. They attributed the difference in active immune response in mice and rabbits to the inability of mice to form somatic antibody. Ribi and associates[32] have found that specific active immunity can also be stimulated with endotoxins in mice against *S. enteritidis*, but

only if antibodies to O antigens were produced. On the basis of these three studies, it is clear that Salmonella endotoxin, by virtue of its antigenic properties, can induce a highly specific active immunity against *lethal* Salmonella infections.

The specific immunizing effect of endotoxin in *sublethal* infections has been studied by Abernathy in murine brucellosis.[25] He observed that 50 per cent of mice immunized with three weekly injections of *B. melitensis* endotoxin completely eliminated a challenging infection of 10^4 *B. melitensis* organisms, while all controls became heavily infected. In the remaining immunized mice that became infected, the bacterial counts of the spleen were considerably lower than those of the controls. The specificity of this immunization is apparent from the fact that mice immunized with endotoxins from *S. typhi*, *E. coli*, and *Shigella sonnei* had no resistance to the sublethal brucella infection.

Conclusions

With few exceptions, the effects of endotoxin on resistance to experimental infection seem to be related to changes in susceptibility to the toxins of the infecting bacteria. Such an exception is found in the experiment with local skin infections; in this instance the deficiency of tissue leukocytes presumably enabled the staphylococci and clostridia to infect larger areas of skin. In most of the remaining infections analyzed in this review, the prior administration of endotoxin probably affected the response to the bacterial toxins. Thus, in many experiments where lethal infections were enhanced, resistance was apparently reduced by the combined effect of two toxic agents administered together. In other experiments, in which resistance to infection was increased by endotoxin, there was good evidence that specific immunity had been induced to the endotoxin possessed by the infectious agent and that local or systemic toxicity was thereby minimized. The most impressive support for this concept is found in the passive transfer of resistance to infection by means of antisera containing specific O antibody. But the reduction of tissue injury without a commensurate fall in the concentration of viable toxic bacteria is also strong evidence that the toxin is neutralized to a certain extent.

The most difficult phenomenon to explain is the brief increase in resistance to infection that occurs shortly after a small injection of endotoxin. The rapid development of such immunity undoubtedly has its

counterpart in human infection and seems to provide a means of body defense against bacterial invasion that is uniquely different from the classical systems of immunity.

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CONSIDERATION OF CERTAIN HOST FACTORS IN THE PATHOGENESIS OF PYELONEPHRITIS*

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THE present state of our knowledge concerning the pathogenesis of pyelonephritis and related infections of the urinary tract has reached a point at which it is proper to critically review certain aspects of these data in order to determine their interrelationships as well as suggest possible future investigative efforts. Particularly important in this regard appear to be those factors which alter host resistance (both systemic and local) to infection. For many reasons, it is desirable to consider clinical and experimental observations separately and then attempt to interpret each in the light of the other.

I. CLINICAL CONSIDERATIONS

Table I lists some of the clinical circumstances which predispose to, or are associated with, a high incidence of urinary tract infection.

A. OBSTRUCTION TO FLOW OF URINE

Obstruction to the flow of urine has long been appreciated to alter the susceptibility of the kidney to infection. This obstruction may be present at any point in the urinary tract from the proximal convoluted tubule to the urethra and still exert this effect. The type of obstructing lesion and its relative frequency of occurrence varies with the age group studied: congenital anomalies in children, pregnancy in early adulthood, tumor in older age groups, calculi and possible metabolic factors in all ages. "Functional obstructions", without evident mechanical lesions, are important and can occur at any age.

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TABLE I—CLINICAL FACTORS ASSOCIATED WITH ALTERED RENAL RESISTANCE TO INFECTION

A. *Obstruction to flow of urine.*
1. Intrarenal. Congenital-developmental anomalies, nephrocalcinosis, gout, K deficiency, Vitamin A deficiency, drugs and chemicals, scarring secondary to previous infection.
2. Extrarenal. Congenital-developmental anomalies, pregnancy, prostatic hypertrophy, tumor, calculi, "functional" alterations.

B. *Vascular abnormalities.*
Renal artery and vein thrombosis, arteritis, (?) nephrosclerosis.

C. *Lymphatic obstruction.*

D. *Miscellaneous.*
Diabetes mellitus, Laennec's cirrhosis, altered antibody production, genitourinary antimicrobial substances, instrumentation.

The mechanism(s) by which urinary obstruction produces this altered resistance is unknown. Speculation has been made concerning the possible role of such factors as increased interstitial pressure, urine stasis, diminished renal blood and/or lymph flow, impaired phagocytosis, metabolic alterations within various renal cells, etc., but there is no definitive clinical or experimental evidence to support any of these hypotheses.

It is apparent that the listing in Table I is not complete nor is it intended to review each of these conditions individually. Rather, it would be of interest to comment about certain of these, particularly as they relate to the common problem of obstruction.

1. *Intrarenal Obstruction.* A wide variety of conditions produce intrarenal anatomical changes which are associated with altered resistance to infection. These include congenital-developmental anomalies, scarring secondary to previous infection, such "metabolic lesions" as nephrocalcinosis, gout, potassium deficiency and possibly administration of certain drugs. In the majority of these circumstances, the lesion produced is a small wedge of intrarenal hydronephrosis secondary to tubular obstruction. The latter may be due to a congenital tubular narrowing, calcium or uric acid deposits, or tubular epithelial changes which result in tubular "plugging" as may be seen in potassium and vitamin A deficiency.[1] There is no evidence that the physiological alterations associated with these lesions *per se* effect renal resistance to

TABLE II—CONGENITAL-DEVELOPMENTAL ANOMALIES WHICH PREDISPOSE THE KIDNEY TO INFECTION

Condition	*Autopsy incidence*	*Incidence of infection*
Aplasia	1:400 to 1:1700	**
General hypoplasia	1:350 to 1:1000	60-70%
Partial hypoplasia	*	increased
Focal hypoplasia or "dysplasia"	*	increased
Polycystic disease	1:300 to 1:1000	50-70%

* Autopsy incidence cannot be determined.

** Incidence of infection increased in involved kidney. In aplasia and agenesis the incidence of infection in the single "normal" kidney is estimated to be 30-40%.

infection. Some drugs, such as phenacetin, appear to produce a chemical interstitial nephritis which may become secondarily infected. The evidence that 4, 4'–diaminodiphenyl sulfone ("Dapsone"–a drug used in treating certain skin diseases) predisposes to infection is not conclusive.

Scar formation secondary to healing or progressive chronicity of infection also results in areas of tubular obstruction and intrarenal hydronephrosis. Clinically, this manifests itself as an increased susceptibility to subsequent re-infection. Experimental confirmation of this was obtained by DeNavasquez[2] who noted the occurrence of Gram-negative bacillary infection in rabbits which had survived preceding staphylococcal infection with resultant areas of healed renal scarring. In these experiments, the active infections were confined to the dilated tubular segments immediately adjacent to the healed scar. Murphy and Schoenberg[3] have suggested that this heightened susceptibility to infection noted after healing of staphylococcal pyelonephritis may be related to intrarenal lymphatic obstruction.

The occurrence of congenital-developmental lesions, whether generalized or focal, constitutes an area of "tissue inferiority" or "*locus minoris resistentiae*". The characterization and incidence of these anomalies have only been partially determined. Table II, adapted from Kleeman and associates,[1] indicates some of the common congenital-developmental anomalies, their general autopsy incidence and the frequency of superimposed infection. Further evidence of the etiologic significance of these factors in the pathogenesis of pyelonephritis may

be noted from: a) the observation that the infectious process was frequently confined to the dysplastic segments of the kidney; and, b) the reversal of sex incidence of pyelonephritis during the first year of life when compared with that after this time and the high rate of occurrence of intrarenal abnormalities in these patients. Thus, while the sex distribution after age one year was 9:1 with females predominating, the ratio was equal or possibly had some male predominance in the neonatal period. When the kidneys of these children with neonatal pyelonephritis were examined, a high incidence of focal or partial dysplasia was noted.

2. *Extrarenal Obstruction*

Pregnancy. The ureteral dilatation associated with pregnancy (due either to ureteral compression by the gravid uterus over the pelvic brim or to hormonal changes associated with pregnancy) has been cited as an obstructive phenomenon which predisposes the kidney to infection. Recently accumulated data[4] suggest that this hypothesis must be re-evaluated and an attempt made to define more critically the role of urinary obstruction in: 1) the intrarenal localization and multiplication of bacteria, and 2) the natural history of symptomatic and asymptomatic disease. It has been noted that the incidence of bacteriuria in gravid patients (6 per cent) is similar to that of nonpregnant females examined at the same hospital. When the incidence of bacteriuria is studied in relation to duration of pregnancy, at first prenatal visit it appears that most, if not all, instances of bacteriuria are acquired before the second month of pregnancy. This is at a time prior to marked demonstrable changes in the urinary tract. In addition, as the ureteral dilatation and obstruction to flow of urine increases with duration of pregnancy, the incidence of bacteriuria does not increase. These facts, plus the observation that there was no difference in volume of residual urine in bacteriuric and nonbacteriuric patients at comparable periods of pregnancy, indicate that the anatomic changes in the urinary tract associated with pregnancy do not account for the appearance of bacteriuria. That these changes may alter the subsequent clinical course of urinary tract infection is suggested by the occurrence of clinically manifest pyelonephritis in about 40 per cent of bacteriuric patients during the last half of pregnancy or shortly after delivery, a circumstance which does not occur when bacteriuria was eliminated by

chemotherapy.

Although pregnancy and pyelonephritis have not been extensively studied in laboratory animals, there are experimental observations which may be considered as counterparts to the urinary tract changes noted. When partial ureteral obstruction was produced in rats and rabbits by: 1) beta irradiation of the ureter; 2) partial ligation; or 3) congenitally occurred; no evidence of increased bacterial localization or multiplication within the kidney could be demonstrated (see Experimental Considerations).

B. VASCULAR ABNORMALITIES

Although there appears to be an association between hypertension, nephrosclerosis and pyelonephritis, this relationship has not been precisely defined. Clinically, the sole demonstration of a high incidence of bacteriuria in hypertensive individuals or the frequent occurrence of hypertension in patients with pyelonephritis leaves doubt as to the etiological relationship therein described. What is needed is information concering the rate of appearance of bacteriuria in otherwise normal hypertensives as well as the frequency of development of hypertension in patients with pyelonephritis when compared with normals. Some data are available which may have bearing on the former group. In 2,949 autopsies performed at the Research and Educational Hospitals of the University of Illinois during 1947 to 1958, the incidence of pyelonephritis among patients with nephrosclerosis (10 per cent) was similar to the average for all autopsies (11.6 per cent).[5] This suggests that clinically, patients with nephrosclerotic disease (which is frequently found in individuals with hypertension) are not more susceptible to kidney infection. In conflict with this is an accumulation of experimental data which suggests that a similar renal vascular disease, whether associated with hypertension or not, predisposes to pyelonephritis. Woods[6] and others[7] have shown that steroid saline-induced hypertension and nephrosclerosis will diminish renal resistance to infection in the rat. Additional observations have indicated that this altered susceptibility to infection will persist when the elevated blood pressure is controlled with hypotensive agents. It must be concluded at the present time, that further studies are necessary before it is possible to define the relationship between renal vascular disease, pyelonephritis and hypertension.

C. LYMPHATIC OBSTRUCTION

There are only limited clinical observations on the role of lymphatics in the pathogenesis of pyelonephritis and these do not permit conclusive definition.[8] To a lesser extent, similar comment may be made concerning experimental approaches to this problem. In the laboratory, it has been demonstrated that: 1) obstruction of lymphatics draining the kidney results in heightened susceptibility to infection; and 2) the lymphatics play little role in the intrarenal spread of infection. There are animal studies which have suggested possible lymphatic channels connecting the lower urinary tract with the kidney and expression has been made concerning the ascent of infection along these pathways.[1]

D. MISCELLANEOUS FACTORS

1. *Diabetes Mellitus.* Urinary tract infections are approximately twice as common in diabetics as compared to nondiabetics. Also, under certain circumstances, these infections tend to be more severe and more protracted in diabetics. Necrotizing papillitis, a fulminating form of pyelonephritis, is more likely to occur in the diabetic.

The reasons for the high incidence of urinary infection in diabetics are not clear. Several hypotheses[1] have been made in this regard: (a) this represents part of the generalized susceptibility to infection commonly ascribed to diabetics; leukocytes of diabetic patients exhibit diminished glycolysis and lactic acid production *in vitro* which can be restored to normal with insulin. This inability to produce lactic acid normally may be an index of diminished resistance to infection; (b) the occurrence of glycosuria provides a more favorable culture medium for bacteria which might gain access to the urinary tract; (c) diabetics with Kimmelstiel-Wilson lesions of the kidney have a higher incidence of pyelonephritis than diabetics without evidence of intercapillary glomerulosclerosis. Whether this complication indicates a more chronic, poorly controlled phase of the disease and hence might be expected to be associated with greater incidence of infection or represents a specific renal factor which predisposes to pyelonephritis has not been established; (d) other observations which might be important are the relatively greater frequency of catheterization for diagnostic purposes in diabetics when compared to normals, as well as the occurrence of diabetic neuropathy which is occa-

sionally associated with disturbances in bladder function. These two findings, whether considered together or separately, can contribute to the colonization and multiplication of bacteria within the urinary tract.

2. *Laennec's Cirrhosis.* Recent observations have suggested that patients with Laennec's cirrhosis may have a higher incidence of urinary tract infections than normals. Tisdale and Klatskin[9] noted that almost one half of cirrhotic patients with proven infection had genitourinary involvement; 10 of 22 of these individuals had cystitis and/or pyelonephritis. In this study urinary tract infection was more common than any other infection. Among 124 autopsied patients with nonobstructive pyelonephritis, hepatic cirrhosis was noted in 11 (9 per cent), as compared with an incidence of 12 per cent of diabetes mellitus in this group.[5] It will be important to determine whether this is a truly higher incidence and, if so, what factors are involved. No more than speculation may be made as to the possible role of altered antibody production, diminished bactericidal activity of serum and/or urine, increased ammonia production and its effect on serum complement, etc. (see Experimental Considerations) in the pathogenesis of urinary infection in these patients.

3. *Altered Antibody Production.* Martin, Bronstein and Dray[10] noted that in patients with agammaglobulinemia "pyelonephritis is very common; their susceptibility almost defies the classic concept of Weiss and Mallory of the pathogenesis of pyelonephritis; they will develop it despite the absence of any obstructive phenomena in the genitourinary tract." Although this observation has not yet been completely confirmed, it may be an extremely important link in understanding the possible role of immune mechanisms in pyelonephritis. As previously mentioned, this type of disturbance may be a consideration in the pyelonephritis associated with Laennec's cirrhosis.

4. *Genito-Urinary Antimicrobial Substances.* Although of unknown significance, a variety of antimicrobial substances have been isolated from kidney, prostate and urine.[1] Early observations by Hain[11] described a distinct bactericidal property of dog urine which was independent of the pH effect and was active against both Gram-positive and Gram-negative bacteria. Boyce and King,[12] in a study of the total nondialyzable solids of urine, noted that certain potentially antimicrobial substances appeared in the urine at various stages of pyelonephritis. Qualitative and quantitative differences in the appearance of these

products in pyelonephritic urine, when compared to other renal disease states, suggest that this may be more than the simple passage of serum products through the diseased nephron and that they may be important in the renal defense against infection. Jackson and Grieble[13] noted a more rapid growth of bacteria in pooled pyelonephritic urine when compared to normal urine. This could represent the absence of, or interference with certain normally occurring microbial inhibitors. The work of Beeson and Rowley[14] (described under Experimental Considerations) may be important in this regard. They noted that renal tissue interfered with the normal bactericidal activity of serum and that this was related to the ammonia inactivation of complement. In the presence of renal damage, complement appears in the urine.[15] Whether alternations in ammonia production associated with renal disease effect any important potential antimicrobial activity of urinary complement is unknown.

5. *Extrinsic Factors.* Instrumentation of the urinary tract undoubtedly plays an important role in the pathogenesis of pyelonephritis. The data which may be marshaled in this regard were editorially summarized by Beeson[16] who stated "The belief is widely held that, with proper precautions, or that by using prophylactic chemotherapy, the risk of infection is negligible, or that at worst, simple cystitis is the only hazard. There is, however, reason to argue that, despite observation of the most careful technic, infection of the bladder not infrequently results from catheterization, and that this can lead to pyelonephritis, a disease of unquestioned gravity."

In support of this argument are the following: (a) Cystitis occurred in 3 to 6 per cent of patients following simple catheterization of urethra; (b) Patients with neurogenic bladder disease and/or poliomyelitis were frequently noted to have sterile urine at time of initial catheterization. With repeated instrumentation or the insertion of an indwelling catheter, infection resulted. A similar situation was noted in a comparison of incidence of infection in patients with upper versus lower urinary tract obstruction. Infection was more frequent in the latter group and this was correlated with a greater use of catheterization; (c) Experiments have been performed which indicated that microorganisms can enter the bladder in patients with indwelling catheters along the muco-purulent sheath that invariably forms between catheter and urethral mucosa; (d) Numerous epidemiological studies of intra-

TABLE III—EXPERIMENTAL FACTORS ASSOCIATED WITH ALTERED RENAL RESISTANCE TO INFECTION

A. *Obstruction to flow of urine.*
   1. Intrarenal. Nephrocalcinosis, uric acid deposits, sulfa drugs, K deficiency, Vitamin A deficiency, scarring secondary to previous infection, mechanical factors (microcautery, needle puncture, etc.).
   2. Extrarenal. Ureteral ligation (complete), *(?)* congenital-developmental anomalies.

B. *Vascular abnormalities.*
   Nephrosclerosis (DOCA-saline induced), renal vein constriction.

C. *Lymphatic obstruction.*

D. *Miscellaneous.*
   Renal massage, anticomplementary activity of kidney.

hospital spread of urinary tract infections have shown that catheters and other similar instruments can act as vectors in the transmission of bacteria to patients. In this regard, instances of hospital-acquired, antibiotic-resistant urinary infections have been described in association with indwelling catheters.

At the present time, the subsequent course of instrument-acquired bacteriuria is not known. Information is not available which would permit estimation of spontaneous "clearing" or persistence of bacteriuria. It would be important to determine which factors might predispose to evolution of infection in these patients. While no one would deny the great diagnostic and therapeutic value of instrumentation, the real implication of potential danger is enough to cause all physicians to weigh carefully all considerations before subjecting the patient to these procedures.

II. EXPERIMENTAL CONSIDERATIONS

The experimental study of pyelonephritis has encountered certain difficulties. While *E. coli* is the causative bacterium in 80 to 90 per cent of patients with acute, nonsurgical pyelonephritis, it has not been possible to produce urinary tract infection in normal laboratory animals by either intravenous, intravesicular or intrapelvic injection of various strains of this organism. Once the kidney is damaged, by one of many techniques, infection readily follows the introduction of bacteria into the urinary tract. Other problems, in addition to this pathogen-host

specie variation have arisen. Nevertheless, certain informative observations have been made in the laboratory and these, when applicable, will be discussed.

Table III contains a list summarizing those experimental manipulations which predispose the kidney to infection.

A. OBSTRUCTION TO FLOW OF URINE

1. *Type of Obstruction*

(a) Complete Ureteral Obstruction. Although *E. coli* does not multiply or produce infection in the normal kidney, acute, complete ureteral obstruction will permit this circumstance to occur. Furthermore, it has been shown that such obstruction effects the various parts of the kidney differently. Thus, following the intravenous injection of *E. coli* into rabbits with ureteral obstruction, Gorrill[17] found that while immediate bacterial multiplication occurred in the medulla there was a 90-minute delay in multiplication in the cortex. Freedman and Beeson[18] also noted a cortico-medullary difference when *E. coli* was injected directly into various portions of the kidney (a manipulation which resulted in obstruction of groups of collecting tubules with subsequent formation of segments of intrarenal hydronephrosis). Under these cicumstances, medullary infection followed the introduction of fewer than 10 bacilli into that area, whereas 100,000 or more were usually required to establish infection in the cortex. These differences in minimum cortical and medullary infecting doses remained essentially unchanged when bacteria were injected into kidneys with ligated ureters.

(b) Partial Ureteral Obstruction. It is difficult to summarize and interpret published data related to the role of partial ureteral obstruction in the pathogenesis of experimental pyelonephritis. This is due to the multiplicity of techniques employed to produce the partial obstruction as well as the recent demonstration that moderate ureteral manipulation, even of relatively short duration, can result in sufficient local traumatic tissue changes to produce complete obstruction.[19] Nevertheless, several pertinent observations are important in this regard. Following the intravenous injection of strains of *E. coli* or staphylococci into rats and rabbits with unilateral partial ureteral obstruction (produced by direct beta irradiation of the ureter and confirmed by intravenous pyelography) the incidence of infection in the hydrone-

phrotic kidney was the same as that noted in the normal organs. Prat, Benesova and Cervinka[20] attempted to produce partial ureteral stenosis by ligating the rabbit ureter over the shaft of a hypodermic needle and then removing the needle. Nineteen of such manipulated animals were given an intravenous injection of *E. coli* and sacrificed three to four weeks later. Histological evidence of pyelonephritis was seen in three animals and "pyelitis" in ten. In only eight of these animals was *E. coli* cultured from the manipulated kidney; an incidence of bacteriologically proven infection of less than one-half of animals tested. Furthermore, it was noted that, "pyelonephritis only developed where the stenosis was very marked or where a complete obstruction of the ureter developed secondarily."

The types of study just described tend to emphasize the problems associated with experimental attempts to produce partial ureteral obstruction. A recently described occurrence of congenital, unilateral, incomplete ureteral obstruction in the Slonaker-Addis strain of white rat has provided a model which more closely resembles the human counterpart and has permitted observation of the relationship between infection and urinary obstruction. In this animal, Sellers, Rosenfeld and Friedman[21] have shown that the incidence of infection in the hydronephrotic kidney following the intravenous injection of a strain of *E. coli* is the same as that noted in the normal organ. In our laboratory, we have confirmed these observations when *E. coli* was injected intravenously into this strain of rat.[22] In addition, experiments employing the intravenous inoculation of a culture of hemolytic, coagulase-positive *Staph. aureus*, an organism of proven nephropathogenicity in the rat, have failed to demonstrate any difference between infection of obstructed and normal kidney.[23] Studies are now in progress to determine whether such a difference can be noted in models of "ascending" infection.

These observations, when viewed collectively, suggest that while complete ureteral obstruction results in increased susceptibility of the kidney of experimental animals to infection, partial obstruction does not uniformly produce this effect. It is not known whether these differences are related to quantitative alterations in intrarenal pressure and its attendant changes in blood and lymph flow, etc., or to qualitative differences related to acuteness of onset of obstruction. Acute, complete obstruction is a traumatic and artificial circumstance which

is associated with sudden and marked changes in renal physiology, while with partial, slowly produced obstruction, alterations in lymph and blood flow may develop to a degree not achieved with abrupt complete obstruction. Indeed, although increased intrarenal pressure occurs in both types of obstruction, there may be considerable differences in the two models.

2. *Duration of Obstruction*

The duration of obstruction prior to the injection of microorganisms is an important determinant of altered renal resistance to infection. It has been shown that ureteral obstruction for as short a period as 15 to 30 minutes is sufficient to effect decreased resistance.[24] Gorrill[17] made important observations in this regard, in his studies of the rabbit –*E. coli* model. In these investigations, it was found that when microorganisms were injected immediately after ligation, rapid bacterial multiplication occurred in both cortex and medulla. However, after more prolonged obstruction, while the medulla still permitted bacterial multiplication, the cortex did not. This latter occurrence represents a return of the cortex towards normal, while the altered medullary susceptibility persists. Similar changes were noted by Freedman, Kaminskas and Beeson[25] using a different model. In rabbits with intrarenal hydronephrosis produced by micro-cautery medullary scarring, the heightened susceptibility of the kidney to infection noted within the first week after trauma returned to normal by the second to fourth month. This change occurred despite continued histological evidence of tubular obstruction.

The importance of this relationship between duration of obstruction and interval of bacterial injection was further emphasized in the following experiments: When a strain of *E. coli* was injected into rats, the microorganisms persisted in the normal kidney in relatively constant numbers up to seven days and then gradually disappeared. If ureteral ligation was performed one day after injection, five of seven animals developed infection while only 3 of 17 became infected when obstruction was delayed until the fifth day.[26] Observations of a similar nature were made in rats with established, chronic *E. coli* infection.[27] In this model, it was noted that the ability to produce recrudescence of healing infection by acute ureteral occlusion decreased in animals studied at three months when compared to six weeks. Therefore, it

would appear that not only does the behavior of the cortex and medulla vary with duration of obstruction but also the "pathogenicity" of bacteria is altered with prolonged residence in renal tissues. The mechanisms by which these changes occur are not known.

B. VASCULAR ABNORMALITIES

1. *Arterial and Arteriolar Disease.* The role which arterial and arteriolar disease may play in the pathogenesis of pyelonephritis has received little attention. Nevertheless, this is an extremely important consideration, particularly as it relates to the association between hypertension and renal infection. Recently, Woods[6] has obtained evidence that rats made hypertensive with desoxycorticosterone trimethylacetate and saline are more susceptible to *E. coli* infection than normal. Furthermore, these investigators demonstrated that this altered renal susceptibility to infection was due to the intrarenal vascular changes (nephrosclerosis) rather than elevated blood pressure *per se*.[28] These experiments have, in part, been confirmed.[7] Laboratory studies of this type have direct bearing on the clinical question of the etiological concepts of hypertension and pyelonephritis and possibly to the syndrome of "malignant hypertension" or "pyelonephritis lenta".

2. *Venous Disease.* Brumfitt and Heptinstall[29] studied the effects of partial renal vein constriction on the course of renal infection in the rat. Their data suggest that the behavior of injected *E. coli* in the kidney with renal vein obstruction is qualitatively similar to that noted in animals with ureteral obstruction. In considering these two models, many alterations are commonly noted. Both are associated with increased intravascular pressure (and elevated tissue pressure) as well as intrarenal vascular congestion and altered renal blood flow. It could be speculated that these two models similarly alter renal lymph flow and other host defensive mechanisms in such a way as to produce increased susceptibility to infection by identical means.

C. LYMPHATIC OBSTRUCTION

While considerable investigative effort has been directed toward determining the importance of lymphatic channels in the movement of bacteria from the intestines and urethra to the kidney, surprisingly little research has been done in evaluating the role of lymphatics in the intrarenal phase of the disease.[1] These studies may be summarized

to state that although there is little evidence that intrarenal spread of infection occurs along lymphatic pathways, extrarenal obstruction to lymph flow results in increased susceptibility of the kidney to infection.[3] Here again, speculation may be made as to whether the intrarenal pressure and other changes which occur secondary to lymphatic obstruction are not common findings to those noted with renal vein and ureteral obstruction and hence similarly account for altered resistance to bacterial infection.

D. MISCELLANEOUS

1. *Mechanical Trauma.* A variety of mechanical injuries, in addition to those associated with obstruction to urine flow, have been used to predispose the kidney to infection. Investigators have employed intrarenal injection of bismuth paste, injection of blue moss seeds into the renal artery, capsule stripping, needle puncturing of parenchyma, a blow to the kidneys, and renal "bruising" in attempts to produce localization of bacteria. In many of these manipulations it is possible to identify factors which might produce areas of increased intrarenal pressure and/or intrarenal hydronephrosis and thus be expected to alter resistance to infection. More recently, Braude, Shapiro and Siemienski[30] described the occurrence of pyelonephritis in rats following renal "massage" and bacterial injection. This procedure results in marked renal hyperemia and congestion and possibly is accompanied by microscopic capillary hemorrhages and thrombi. Whether the altered resistance to infection described in this model is a reflection of similarities to the changes noted following renal vein obstruction or represents another method of interfering with antibacterial defenses is not known.

Such "metabolic" lesions as nephrocalcinosis, uric acid deposits, sulfa drug nephropathy, and potassium deficiency represent mechanical injury to the kidney in that all of these cause tubular obstruction and intrarenal hydronephrosis. It is this physical consequence, rather than the physiological alterations, which predisposes the kidney to infection.

2. *Biochemical Factors.* Experimental attempts to define a metabolic or biochemical abnormality which might render the kidney more susceptible to infection have been largely unsuccessful with one notable exception. Beeson and Rowley[14] in a very fine study obtained evidence which indicated that renal tissue was able to interfere with the normal bactericidal activity of serum. Furthermore, it was shown that this

effect was related to the anticomplementary activity of kidney. The component of complement affected was the fourth; i.e., that which is characterized by susceptibility to injury by ammonia. Freedman[31] presented data which confirmed these findings in the experimental rat—*E. coli* infection. There are several clinical observations which would indicate that this finding is important. Rantz[32] in a study of susceptibility of various strains of *E. coli* to serum bactericidal activity noted, "It is clear that the substances responsible for the serum effect do not kill Gram-negative bacilli in the kidney although they seem to have a definite role in elimination of organisms from the circulating blood." Another possible manifestation of this may be the finding by Tisdale and Klatskin[9] that of 22 patients with Laennec's cirrhosis and proven infection, ten had evidence of genitourinary infection. This is a group in which elevated blood ammonia occurs not uncommonly. Data have been presented which demonstrate the occurrence of antibacterial factors in urine.[12] It appears as though many of these substances are derived from the kidney and/or prostate. In addition, when proteinuria is present, components of the complement system and specific isohemagglutinins appear in the urine.[15] It is possible that these factors play a role in protecting the urinary tract from infection and may be interfered with under circumstances of high serum and/or urine concentrations of ammonia.

Comments

This consideration of certain host factors in the pathogenesis of pyelonephritis has emphasized the role of obstructive uropathies in this disease. Some of the experimental observations cited have indicated that partial, slowly produced obstruction to the flow of urine does not alter the immediate fate of bacteria within the kidney. Some clinical support of this may be noted in studies of urinary infections associated with pregnancy. This concept is at variance with many classically held hypotheses and requires further comment. While many of the changes associated with ureteral obstruction are unknown, it would appear as though there are considerable qualitative and quantitative differences between acutely induced complete obstruction and the gradually produced partial constriction. Furthermore, compensatory changes of blood and lymph flow occur and these can obviously effect profound alterations in the physiological and anatomical environment of the ob-

structed kidney. The importance of these changes was seen in the several experiments which described a return toward normal resistance to infection as the duration of obstruction was prolonged. Thus, it becomes apparent that in considerations involving the concept of obstructive uropathy and infection, awareness of the anatomical and functional state of the kidney (a reflection of induced and compensatory changes) is of great importance. Unfortunately little knowledge is available concerning these factors. It is therefore suggested that this is an investigative area which should be entered if a better understanding of the pathogenesis of urinary infections is sought.

The effects of prolonged host-parasite interrelationships on bacteria as well as the host are being recognized as important, although as yet poorly defined, facts in this problem. The ability of microorganisms to persist within the urinary tract for prolonged periods of time and apparently lose some of their "nephropathogenic" qualities and alternatively enhance these same qualities under similar circumstances (i.e., the appearance of clinically manifest pyelonephritis at time of delivery in pregnant patients who had asymptomatic bacteriuria for many months) is difficult to understand and requires further study.

A final comment is concerned with the role of the pathway of infection. There are several observations in the literature which suggest a difference in susceptibility of the kidney to infection depending on whether an intravenous or intravesicular route for introduction of bacteria is used. It is not clear whether this is related to the portion of kidney initially invaded when the various routes are employed or is a reflection of continuous "bathing" of the kidney by bacteria multiplying within the urine. It would be of interest to determine whether or not this is significant in a model employing both routes of infection in animals with partial ureteral obstruction. These may be more than academic considerations since asymptomatic bacteriuria (which has been identified with the potential subsequent development of renal infection) can be diagnosed and eliminated.

Summary

Certain aspects of the role of host factors in the pathogenesis of pyelonephritis have been discussed. Clinical and experimental observations concerned with the etiological significance of extrarenal obstruction to the flow of urine have been presented. These data indicate that

some of the clinically accepted hypotheses are at variance with recently accumulated information and it is suggested that additional experimental efforts be made in an attempt to identify those factors which might account for these differences.

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VASCULAR EFFECTS OF ENDOTOXINS*

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IN Table I are listed some of the more striking biological phenomena produced by injections of bacterial endotoxins. It has become increasingly clear over the past two decades that many of these phenomena are mediated by vascular reactions of one sort or another. The pyrogenic effect of small doses of endotoxin and the shock produced by larger doses are both associated with profound vasomotor changes.[1] The local reaction to injections of endotoxin is characterized by striking alterations in the local blood vessels.[2] The dermal Shwartzman reaction, in which a large area of skin exhibits a peculiar sort of hemorrhagic necrosis, has been shown to depend on a sequence of changes in local blood supply,[2] while the so-called generalized Shwartzman reaction likewise is due to severe blood vessel damage and occlusion.[3] The hemorrhage and necrosis observed in experimental tumors after intravenous injection of endotoxin appears to be secondary to vascular damage[4] as do several other effects of endotoxin. The purpose of this paper will be to examine what is now known of the vascular effects of endotoxin, and to indicate to what extent these effects may constitute common pathogenetic mechanisms in the various phenomena produced by endotoxin.

In Table II are listed some of the well-established effects that need to be considered. All of these effects can be demonstrated within minutes or hours after the injection of endotoxin, and nearly all have been demonstrated in several species of animals, including man, so that they may be regarded as characteristic and potentially significant components of the overall host response. It would be misleading to suggest that all of the important vascular effects of endotoxin have been identified, but this table indicates some that may be involved in the mechanism of the phenomena listed in Table I, and these will be considered in turn.

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TABLE I—SOME PHENOMENA PRODUCED BY ENDOTOXIN

1. Fever
2. Shock
3. Dermal Reaction
4. Shwartzman Phenomena:
   (a) Local Hemorrhagic Necrosis
   (b) Bilateral Renal Cortical Necrosis
5. Tumor Hemorrhage
6. Abortion
7. "Toxic Ocular Reaction"
8. Increased Resistance to Shock ("Tolerance")
9. Increased Antibody Response ("Adjuvant Effect")
10. Increased Resistance to Infection (Nonspecific)

TABLE II—SOME "VASCULAR" EFFECTS OF ENDOTOXIN

1. Vasomotion
2. Increased Vascular Permeability and Stickiness
3. Hyperreactivity to Epinephrine
4. Hyporeactivity to Epinephrine
5. Leucopenia and Thrombocytopenia
6. Intravascular Agglutination of Leucocytes and Platelets
7. Thombosis
8. "Heparin-Precipitable" Fibrinogen and "Fibrinoid"
9. Altered Histidine Decarboxylase Activity
10. Fibrinolysis

The vasomotor changes that have been described include a profound peripheral vasoconstriction that begins abruptly several minutes after an intravenous injection of endotoxin and lasts from several minutes to an hour or more, depending on the dose of endotoxin given.[1] The vessels then usually undergo dilation and the muscular arteries of the rabbit's ear, for example, may exhibit segmental vasomotion for some time thereafter. These changes are said not to occur in the denervated rabbit's ear, and can be largely prevented by the administration of adrenergic blocking agents.[1] In a thorough series of investigations, Zweifach and his collaborators[5-7] have shown that these vasomotor effects of endotoxin are apparently due to a biphasic alteration in sensitivity of vascular smooth muscle to epinephrine, small doses of endotoxin rendering vessels extremely sensitive to epinephrine *in vivo* and in the isolated perfused rabbit's ear as well, while with larger doses of endotoxin the epinephrine sensitivity first increases far above normal, then declines to a point where the vessels cannot be stimulated to contract even with enormous amounts of epinephrine. There are many reasons for believing that this altered reactivity to epinephrine is a basic and meaningful feature of the vascular response to endotoxins.

Some years ago it was shown that local intradermal injections of endotoxin lead to the development of delayed inflammatory reactions, resembling the "tuberculin" type of allergic reaction.[2] When epinephrine is infiltrated into such lesions, massive hemorrhage and necrosis ensue, while normal skin suffers no irreversible damage when injected with the same amount of epinephrine. Thomas subsequently showed[8] that the intradermal injection of a mixture of endotoxin and epinephrine causes severe local hemorrhagic necrosis, and that still another combination, endotoxin injected intravenously followed by epinephrine injected intradermally, produced similar local tissue damage. These phenomena all reflect an altered reactivity of blood vessels to epinephrine, but the mechanism of the tissue damage in each case is not yet entirely clear.

The production of generalized increased vascular permeability and endothelial stickiness is apparently another effect of endotoxin. Experiments with transparent chambers in rabbit's ears have shown that intravenous injections of endotoxin are followed by the adherence of leucocytes to capillary and vein walls. This is clearly a complicated phenomenon, both the leucocytes, as isolated from the peripheral blood,[9] and vascular endothelium, as tested separately,[10] are sticky. This generalized stickiness of vascular endothelium and of leucocytes and platelets is a really dramatic feature of the systemic reaction to endotoxins. Clumps of agglutinated leucocytes and platelets can be seen in the peripheral blood stream, and the profound leucopenia and thrombocytopenia which develop are due to the trapping out of these sticky masses in peripheral capillary beds, paricularly in the lung.[2]

The cause of the stickiness has been investigated by biophysicists, immunologists and others, and there is no general agreement as to its cause or nature. One of the most venerable and still popular concepts is that somehow the clotting mechanism is involved, the stickiness being due to a surface coating of fibrin or altered fibrinogen. That endotoxin can initiate and accelerate the clotting of whole blood *in vitro* has been clearly demonstrated[11] and alterations of the clotting mechanism *in vivo* have also been found. Perhaps the most striking such alteration is the demonstration by Thomas and collaborators[3] that after intravenous injection of endotoxin much of the circulating fibrinogen is so altered that it precipitates in the cold in the presence of heparin. This precipitation is reversible, and the altered fibrinogen can also be precipitated by admixture with other acidic polysaccharides. Many animals find some

way of laying down this material as an amorphous "fibrinoid" deposit in glomerular capillaries. This happens more frequently after the second of two intravenous injections of endotoxin, whereupon nearly every glomerular capillary is completely occluded by the material. This obstruction to blood flow naturally causes infarction of the renal cortex, or bilateral renal cortical necrosis. This phenomenon in animals is usually called the "generalized Shwartzman phenomenon", but it is probable that the same mechanism of tissue damage is at work in human cases of bilateral cortical necrosis.

The more classical dermal Shwartzman phenomenon involves quite another mechanism of tissue damage. Here, too, the vascular effects of endotoxin are primarily involved, but in a different way. This phenomenon is produced by the intradermal injection of endotoxin in a rabbit, followed by a period of waiting. During this time an inflammatory response slowly develops, reaching its peak at about 24 hours. A second injection of endotoxin, given intravenously this time, causes a dramatic series of events. Petechiae and purpuric spots develop in the involved skin area, which rapidly undergoes hemorrhagic necrosis.[12]

Much work has been done to provide an understanding of this deceptively simple phenomenon, as it seems to many to provide a useful experimental model for the study of tissue damage in certain human infectious diseases. It has been shown that thrombosis is involved, since the phenomenon can be inhibited by anticoagulant therapy.[13] It has been shown that vasomotor reactions are involved, since the phenomenon can be reproduced by injecting vasoconstrictor agents into the prepared skin site[2] and can be blocked or modified by adrenergic blocking agents. It has been shown that polymorphonuclear leucocytes are involved, since treatment with x-ray or radiomimetic drugs prevents the phenomenon under circumstances clearly implicating these cells.[14] The mechanism seems to be no more complicated, however, than a simple combination of some of the vascular effects already discussed. The intradermal injection leads to an inflammatory reaction in which the walls of small veins become sticky. The intravenous injection produces vasoconstriction, sluggish blood flow and plugging of these vessels by masses of leucocytes and platelets. The complete cessation of blood flow results in death of the tissue, which has demanding metabolic properties. When the animal recovers from the peripheral circulatory collapse and pumps blood into this dead tissue, massive extra-

vasation through necrotic vein walls occurs and the full-blown picture of hemorrhagic necrosis is seen.[2]

The phenomenon of induced hemorrhage and necrosis in tumors, brought about by endotoxin, seems to have a similar basis. Here the tumor, with a glycolytic metabolism closely resembling that of inflamed tissue, is equally dependent for survival on an adequate blood supply. When an intravenous injection of endotoxin is given, vasoconstriction occurs, endothelium of tumor vessels becomes sticky and traps leucocytes and platelets until the vessel is actually occluded by a "white thrombus". Death of the tumor, with subsequent hemorrhage into the necrotic tissue, proceeds as before. It is possible that some of the reactions to Coley's toxin a generation ago were due to this mechanism.

These are some of the more drastic phenomena produced by endotoxin, and they quite obviously involve vascular reactions of a complicated nature—vascular endothelium and vascular smooth muscle are very intimately involved in most of these reactions, while intravascular stickiness and aggregation of leucocytes and platelets on the one hand and fibrinogen and perhaps other clotting factors on the other are conspicuously involved in one or another of them. I have deliberately avoided the subject of shock because, while endotoxin in modest amounts will indeed produce irreversible shock and death in many species, there is at the moment a lively controversy over the subject of whether endotoxins from intestinal bacteria are involved in hemorrhagic or traumatic shock. An adequate treatment of this problem is outside the scope of this presentation, although to be sure, the production of shock is among the most dramatic of the vascular effects of endotoxin.[1, 5-7]

There is little to be gained at the moment by discussing the mechanism by which intravenous injections of endotoxin produce striking changes in the eye of the rabbit[15] or abortion by the pregnant mouse.[16] While there is not doubt that vascular effects are involved in these phenomena as well, it would, perhaps, be more to the point to raise questions concerning the mechanism by which endotoxin induces vascular effects in the first place.

Does endotoxin have a direct effect on vascular smooth muscle, on endothelium or on leucocytes or platelets? The answer seems to be, in each case, that it does not. Each of the vascular effects that we noted in Table II is apparently itself a secondary effect, resulting from some

TABLE III—SOME DIRECT IN VITRO EFFECTS OF ENDOTOXIN

1. Initiation of Coagulation
2. Stimulation of Leucocyte Respiration and Glycolysis
3. Sensitization to Epinephrine

basic biological activity of which we now know almost nothing. So that, although we can explain the phenomenon of renal cortical necrosis, for example, in terms of vascular effects (vasoconstriction, formation of "heparin-precipitable" fibrinogen), we are at a loss in turn to explain how endotoxin produces vasoconstriction or "heparin-precipitable" fibrinogen. Some few effects of endotoxin have been demonstrated *in vitro* (Table III) but in each case again appear to be indirect effects. The effect on coagulation is apparently mediated by natural antibodies, since it can be mimicked by other antigen-antibody complexes and cannot be produced in the absence of antiendotoxin antibodies.[11] The stimulation of leucocyte metabolism in shed whole blood[17] is similarly antibody-dependent. In fact, it has been suggested that perhaps the biological activity of endotoxin may be entirely immunological in nature; that is, that endotoxins represent a class of bacterial antigens so ubiquitous that mammals are "naturally" hypersensitive to them.[18] While there is a good deal to be said for this point of view, there are some telling arguments against it. For example, the chick embryo is easily killed[19] by microgram quantities of endotoxin (death occurs as a result of extensive and severe vascular damage, incidentally) and the newborn rabbit is fully susceptible to the lethal and some of the other effects of endotoxin.[18] Endotoxin can hardly be acting in these cases as an antigen to which the animal is hypersensitive, as we now conceive of hypersensitivity. Work on the biochemistry and toxicology and immunology of endotoxins, however, is almost certain to provide us in the foreseeable future with the insight that we need to understand these vascular effects of endotoxins.

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ROLE OF ENDOTOXIN IN TYPHOID FEVER*

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THE mechanisms by which *Salmonella typhosa* mediates the clinical manifestations and pathologic alterations of typhoid fever are unknown. In 1898, F. B. Mallory concluded: "The typhoid bacillus produces a mild diffusible toxine, partly within the intestinal tract, partly within the blood and organs of the body. This toxine produces proliferation of endothelial cells which acquire for a certain length of time malignant properties. The new-formed cells are epithelioid in character . . . and are characterized by marked phagocytic properties."[1] Evidence has accumulated subsequently to suggest that *S. typhosa* produces other features of typhoid disease by virtue of the endotoxic (somatic or O antigenic) component. This evidence, based upon certain similarities of host alterations during typhoid fever to those observed following intravenous administration of purified *S. typhosa* endotoxin, can be considered as follows:

Symptomatology. Many of the symptoms encountered in patients with typhoid fever can be reproduced in man by the injection of microgram quantities of the purified endotoxin component. The intravenous injection of 0.5 μg. of *S. typhosa* endotoxin** into three healthy male volunteers during studies now in progress at the University of Maryland School of Medicine was followed by headache, chills, fever, myalgia, malaise, abdominal discomfort, and anorexia. These subjects were subsequently infected by feeding 10^6 to 10^9 viable *S. typhosa* organisms, originally isolated from the bile of an asymptomatic carrier.

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Certain of these studies are being supported by the Commission on Epidemiological Survey of the Armed Forces Epidemiological Board as a part of a planned program to evaluate the prophylactic efficacy of typhoid vaccine.

**The endotoxin preparation was provided by Dr. Maurice Landy of the National Institutes of Health, Bethesda, Md.

During overt typhoid infection, the subjects stated that their symptoms were remarkably similar to those induced by the purified endotoxin.

Pathologic findings. Typhoid fever is characterized by hypertrophy of lymphoid tissue and by focal necrosis in liver, spleen, cardiac muscle, bone marrow, and lymphoid follicles.[2] These lesions are diffuse and are not correlated with the presence of living bacteria. This latter feature led Mallory to state "Histologically the typhoid process is proliferative and stands in close relationship to tuberculosis, but the lesions are diffuse and bear no intimate relation to the typhoid bacillus, while the tubercular process is focal and stands in closest relation to the tubercle bacillus."[1] Moreover, the pathologic alterations observed in man during typhoid fever can be reproduced in the rabbit by the intravenous administration of the purified endotoxin component.[3]

Hematologic findings. These are characterized during typhoid fever by polymorphonuclear leucopenia and anemia.[2] Such hematologic changes can be induced with the purified endotoxin component.[4] Some investigators have indicated that the leucopenia of typhoid fever fails to support the concept of endotoxin pathogenesis since leucopenia after the intravenous injection of endotoxin is transient and is followed by leucocytosis.[5] This argument is valid only after single intravenous injections of endotoxin; the effect of continuously infused endotoxin (presumably the manner in which endotoxin is released during typhoid fever) on leucocyte count is unknown.

Serologic findings. During the early phase of typhoid fever, *S. typhosa* endotoxin (somatic antigen) has been detected in the serum by means of precipitin tests employing antibody against the somatic antigen. Subsequently, as host antibody titers to somatic antigen increased, the circulating precipitable somatic antigen disappeared.[6]

Immunologic findings. The appearance of antibodies against *S. typhosa* endotoxin is not correlated with clinical remission.[2] This parallels the observation that antibodies fail to neutralize the toxic activity of bacterial *endotoxins* in the manner that bacterial *exotoxins* are neutralized.[7]

Response to therapy. The response of patients with typhoid fever to administration of cortisone is dramatic. Toxicity clears within hours and the fever is reduced rapidly.[8] This antitoxemic activity of cortisone has been shown during previous[9] and present studies in man to extend to bacterial endotoxin. Such effects may be related to the protective

influence of cortisone on the disturbances in carbohydrate metabolism induced by endotoxin[10] or to the ability of cortisone to mitigate endotoxin-induced tissue injury as determined from measurements of serum transaminase levels.[11] In contrast, the response to antibiotic therapy in typhoid fever is gradual.

It is emphasized that the above evidence linking the clinical characteristics of typhoid fever with endotoxemia is circumstantial, since many similar alterations occur in man during infection with nonendotoxin-containing microbes. The serologic evidence of *S. typhosa* endotoxin in the plasma during the early phase of typhoid fever does not confirm its direct relationship to production of toxic changes.

Endotoxin tolerance. Unfortunately, there are few studies which evaluate critically physiologic alterations during human typhoid infection which could be attributed to the activity of *S. typhosa* endotoxin. Perhaps the most definitive of such studies are those concerned with the phenomenon of acquired tolerance to the pyrogenic activity of endotoxin. If *S. typhosa* endotoxin participates in the pathogenesis of typhoid fever, it appears reasonable that tolerance to endotoxin might be demonstrable during the course of illness. Heyman and Beeson[12] noted that, with the exception of malaria, subjects convalescent two to three days from a variety of infectious illnesses failed to exhibit tolerance to the pyrogenic action of a standardized intravenous inoculum of heat-killed *S. typhosa*. Two subjects in this group convalescent from naturally acquired typhoid fever exhibited exaggerated febrile reactions, i.e., manifested *decreased* tolerance to endotoxin. Neva and Morgan[13] reported the results obtained in 22 patients convalescing from naturally acquired typhoid fever. A significant *increase* in tolerance (mean fever indices 50 per cent below normal) to the pyrogenic activity of *S. typhosa* and of *Shigella dysenteriae* endotoxins was observed upon testing during the fourth to sixth convalescent day. In the above studies, tolerance to endotoxin following typhoid fever was judged from responses obtained in groups of control subjects; variations in febrile responses to the same endotoxin within these control groups were considerable, over six-fold differences existing between the highest and lowest "normal" indices. Moreover, when the data of Neva and Morgan are analyzed critically, six of the twenty-two patients convalescing from typhoid fever exhibited fever indices higher than the mean of the control group, and two of these six patients exhibited fever

indices equal to or higher than the maximum level seen in the control group. The evidence for development of tolerance to endotoxin during typhoid fever, therefore, cannot be considered decisive.

Studies are now in progress at the University of Maryland School of Medicine to re-evaluate the development of endotoxin tolerance during typhoid fever. The fever index following the intravenous injection of purified *S. typhosa* or of *Escherichia coli* endotoxin was determined in healthy male volunteers prior to oral infection with 10^6 to 10^9 viable *S. typhosa*. After several days of illness, the subjects were treated with chloramphenicol and were retested with endotoxin on the third afebrile day. The data, though preliminary, indicate the development of significant tolerance to the pyrogenic activity of *S. typhosa* and of *E. coli* endotoxin during the convalescent phase. These results do not appear to be attributable to the residual refractory effects induced by the control testing with endotoxin, since control subjects tested at similar intervals did not exhibit tolerance. Such findings contrast with those in brucellosis in which convalescent patients developed fever indices upon testing with brucella endotoxin higher than those of control subjects.[14] Moreover, no alteration in endotoxin tolerance has been detected in man following tularemia, gonococcal arthritis, bacillary dysentery, and *Salmonella* gastroenteritis[12, 13] or in rabbits following *E. coli* peritonitis.[15] The demonstration of endotoxin tolerance after typhoid fever, therefore, is apparently unique and does not appear to constitute a general feature of infection with endotoxin-containing microbes. The role of endotoxin tolerance in the recovery process from typhoid fever and the precise time of its appearance in man are currently under investigation.

Vascular reactivity to catecholamines. One other investigative approach to the role of endotoxin in the pathogenesis of typhoid fever is worth consideration. In experimental studies in the intact rat and rabbit, the intravenous injection of bacterial endotoxin was found to induce hyper-reactivity of the peripheral vascular system to epinephrine and norepinephrine.[16-19] The mechanism of action of the endotoxin is as yet unknown. If *S. typhosa* endotoxin participates in the pathogenesis of typhoid fever, it seems reasonable that vascular hyper-reactivity to catecholamines might also be demonstrable during human illness. Studies are now in progress to evaluate this parameter. The subjects employed were those described in the previous section. Responsiveness of systemic

arterial blood pressure and of nailfold capillary blood flow to intravenous norepinephrine infusions were monitored during the pre-infection period, during overt illness, and during convalescence. Preliminary studies indicate the appearance of hyper-reactivity of the vascular system to norepinephrine during typhoid fever which persists into convalescence and subsides by the second to third week after defervescence. These findings raise the possibility that such vascular hyper-reactivity contributes to the genesis of the focal necrosis in the intestine and other organs during typhoid fever.

In summary, the evidence that the endotoxin component of *S. typhosa* mediates the various manifestations of typhoid fever in man has been reviewed. It is emphasized that the data are circumstantial and hence, inconclusive. Studies on typhoid fever in man are now in progress which indicate the development of tolerance to the pyrogenic activity of endotoxin and the appearance of hyper-reactivity of the vascular system to catecholamines. Such findings support the hypothesis that the clinical derangements during human typhoid infection are the direct consequence of the activity of the endotoxin component of *S. typhosa*. The development of tolerance to endotoxin during typhoid fever appears unique and contrasts with observations following infection with other endotoxin-containing microbes. The role of such endotoxin tolerance in the recovery process from typhoid fever is speculative and is currently under investigation.

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SALMONELLOSIS: CERTAIN FACTORS INFLUENCING THE INTERACTION OF *SALMONELLA* AND THE HUMAN HOST*

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THE incidence of human infection with members of the genus *Salmonella* other than *S. typhosa* has increased four- to seven-fold in the past decade.[1, 2] The increase in salmonellosis is probably related to an actual increase in number of cases as well as to improvements in diagnosis and reporting. Although human carriers or persons with subclinical infection are capable of transmitting infection, the greatest source of human disease in the United States at the present time is the remarkable reservoir of *Salmonella* in animals.[1-4] All domestic and many wild animal species are capable of harboring *Salmonella*, with infection rates varying from less than 1 to more than 40 per cent.[2] Uncooked or inadequately cooked food products of animal origin often contain viable *Salmonella* and it is not surprising that previously uninfected food may be contaminated during processing or that by-products of the meat packing industry frequently harbor *Salmonella*. Abundant additional evidence supports the view that *Salmonella* are widespread in the environment and not infrequently contaminate food or drink for human consumption.[2] In view of the prevalence of *Salmonella*, it seems inevitable that man comes in contact with these organisms at relatively frequent intervals.

The outcome of the human host-*Salmonella* interaction depends on the dose of *Salmonella* ingested, or more specifically, the number of viable organisms reaching the small intestine, the characteristics of the organism, and the status of the host. Although detailed knowledge of these factors is lacking, the importance of each has been documented

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for *Salmonella* infection of man.

The studies of McCullough and Eisele[5-7] have demonstrated quite definitely the importance of dose and type of organism. These investigators fed healthy human volunteers graded doses of six species of *Salmonella* isolated from spray-dried whole egg powder, and observed impressive differences in pathogenicity between *one* species and *another*, as well as marked differences in pathogenicity of different strains of the *same* species. Although there were occasional exceptions, with the 13 strains of the six species studied by McCullough and Eisele,[5-7] large numbers (approximately 10^6) of organisms were required to regularly produce disease. However, a transient carrier state was often induced with 10- to 100-fold less organisms than required to produce disease.

Although the factors that influence the outcome of naturally-occurring *Salmonella* infection of man are difficult to dissect, it is clear that certain *Salmonella* species are consistently associated with severer illness than others.[3-8] For example, *Salmonella anatum* usually produces inapparent infection or gastroenteritis and only rarely invades the blood stream.[4] In contrast, *S. choleraesuis* is the most common cause of *Salmonella* bacteremia and infrequently produces subclinical or enteric infection.[4, 8-10]

The literature is replete with observations on the influence of a variety of factors on resistance and susceptibility of animals to experimental *Salmonella* infection.[3] However, there are relatively few studies that attempt to define the importance of host characteristics in the outcome of salmonellosis in man. Several years ago the cases of sporadic *Salmonella* infection admitted to the Johns Hopkins Hospital over a period of years were studied to gain additional information on the role of host factors in human salmonellosis,[11] and recently, similar observations were made at The New York Hospital. The observations at each institution were quite similar and these data have been consolidated. The total number of cases is 305, of which 195 were observed at The New York Hospital from 1940 to 1960 and 110 at the Johns Hopkins Hospital from 1936 to 1958. There were 75 Negroes, 230 whites, 187 males and 118 females.

It is of interest that a major underlying disease was present in 36 per cent (64) of the 178 patients with *Salmonella* gastroenteritis and 40 per cent (52) of the 127 patients with bacteremia, paratyphoid

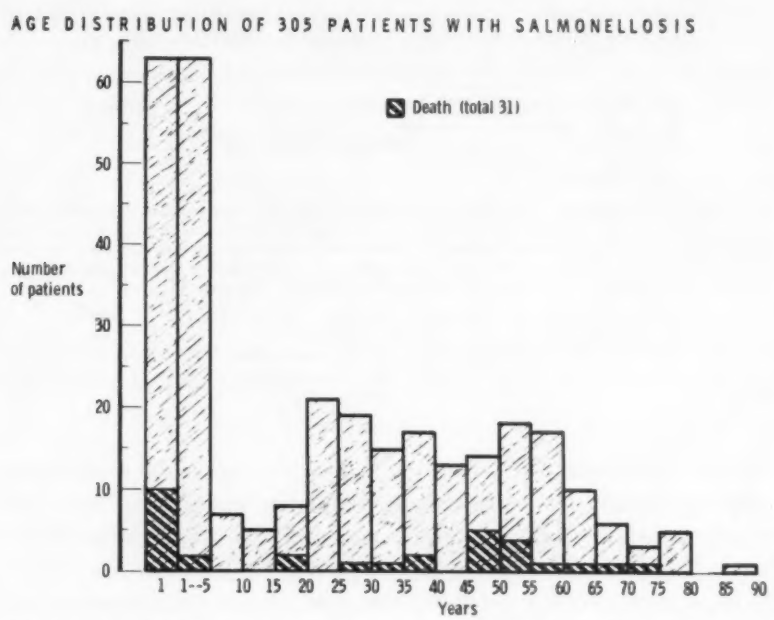

Figure 1—Age distribution and number of deaths in 305 hospitalized patients with *Salmonella* infections.

fever, or localized suppurative infection. If the 32 patients with paratyphoid fever are excluded, the incidence of underlying disease in patients with bacteremia or localized infection was 50 per cent. In another study of hospitalized patients with salmonellosis Eisenberg, Palazzolo and Flippin[10] reported coexisting disease in 74 per cent of adults and 27 per cent of children but gave no additional information.

The age of the patient and the presence of underlying disease appeared to influence the occurrence and course of salmonellosis. As shown in Figure 1, the number of patients less than one year old greatly exceeded the number observed for any other year of life, and 40 per cent of the patients were in the first five years of life. The disproportionately large number of infants and young children as compared to older children or adults could reflect greater contact with *Salmonella* or increased susceptibility to infection or disease. The relative importance of these factors is unknown. However, it has been demonstrated that attack rates for clinical illness are higher in infants than adults.[1, 12-14]

TABLE I—UNDERLYING DISEASES OF THE GASTROINTESTINAL TRACT IN PATIENTS WITH SALMONELLA ENTERITIS

Subtotal gastrectomy, gastroenterostomy	9 patients
Extensive carcinoma of stomach	1
Extensive lymphosarcoma of stomach; achlorhydria	1
Atrophic gastritis; achlorhydria	1
Celiac disease	3

One each: Carcinoma of esophagus, esophagogastrostomy, splenectomy; regional enteritis; atresia of small intestine; partial jejunal obstruction; nontropical sprue; ulcerative colitis; amebiasis; diverticulosis of colon and hemmorrhage; diverticulosis of colon and arthritis; carcinoma of rectum; colostomy and carcinoma of cervix; chronic pancreatitis and diabetes mellitus; chronic pancreatitis and cirrhosis of liver.

Number of patients with gastroenteritis	178
Number of patients with underlying disease	64
Number with underlying disease of gastrointestinal tract	28

Another difference in response that is related to age is the tendency of infants to remain carriers for many months in contrast to adults who usually cease shedding organisms within a few weeks after infection.[3, 13, 14] The mortality rate is also higher in infants than in adults. In the present series, the fatality rate was 16 per cent in infants less than one year old, as compared to 8 per cent in older children or adults. The overall mortality rate was 10 per cent.

Twenty of the 31 patients who died (Figure 1) with coexistent *Salmonella* infection had a major underlying disease. However, only two of the ten fatal cases that were less than one year old had a recognizable basic disease, whereas 18 of 21 patients in the older age groups had a major basic process.

Diseases of the gastrointestinal tract were the most common underlying processes in patients with *Salmonella* gastroenteritis, whereas diseases of the hemopoietic system dominated the group of basic disturbances in patients with bacteremia, localized infection or paratyphoid fever. The underlying diseases of the gastrointestinal tract observed in patients with *Salmonella* enteritis are shown in Table I. The relatively large number of patients with prior subtotal gastrectomy and gastroenterostomy is of interest and is clearly out of proportion to the anticipated incidence of patients of this type. This observation supports the contention of Waddell and Kunz[15] that gastrectomy, gastroenterostomy, and/or vagotomy predispose to *Salmonella* enteritis. Many of the other diseases listed in Table I are characterized by disturbances of small

TABLE II—UNDERLYING DISEASES OF HEMOPOIETIC SYSTEM IN PATIENTS WITH SALMONELLA BACTEREMIA, LOCALIZED INFECTION OR PARATYPHOID FEVER

Sickle cell anemia	6
Sickle cell-thalassemia	2
Thalassemia major	2
Sickle cell-hemoglobin C	1
Sickle trait; hemolytic anemia	1
Acquired hemolytic anemia	1
Nutritional or iron deficiency anemia	3
Acute leukemia	1
Chronic lymphatic leukemia	1
Hodgkin's Disease	1
Lymphosarcoma	1
Number of patients with bacteremia, localized infection or paratyphoid fever	127
Number of patients with underlying disease	52
Number of patients with underlying disease of hemopoietic system	20

and large bowel function, and it is possible that a diagnosis of salmonellosis in these patients represented more efficient detection of infection because of frequent bacteriologic examination of stool. However, in the majority of these patients the episode of acute bacterial enteritis could be clearly differentiated from the underlying disturbance. A more reasonable explanation is that even mild episodes of bacterial enteritis in patients with underlying diseases of the intestinal tract are associated with profound functional disturbances for which medical assistance is required.

Table II shows the underlying diseases of the hemopoietic system in patients with bacteremia, paratyphoid fever or localized *Salmonella* infections. It is remarkable that of a total of 127 patients (50 Negroes) with severe systemic infections, there were ten with major disorders related to the presence of sickle hemoglobin (Table II). Four of the six patients with sickle cell anemia and both patients with sickle cell-thalassemia had osteomyelitis. The occurrence of six cases of sickle cell anemia among 50 Negro patients with *Salmonella* bacteremia, paratyphoid fever or localized infection exceeds 70-fold the anticipated incidence (1:600) of sickle cell anemia in the Negro population of the United States.[16]

A great variety of additional underlying diseases involving all organ systems was encountered in the 305 hospitalized patients with sporadic salmonellosis. In most of these situations the significance of the asso-

ciation between basic disease and *Salmonella* infection is obscure. However, the frequency of salmonellosis in patients with prior major gastric surgery or certain hemoglobinopathies indicates more specific relationships, which are discussed in subsequent paragraphs. The role of antimicrobials in altering susceptibility of man to *Salmonella* infection is also discussed in an additional section.

Salmonellosis in Patients with Previous Major Gastric Surgery. In 1956 Waddell and Kunz[15] first suggested that patients with previous major gastric surgery are more susceptible to *Salmonella* infection than persons with normal gastrointestinal tracts. These investigators observed 34 patients with *Salmonella* gastroenteritis in a period of two years at the Massachusetts General Hospital and found that nine, or 26 per cent, of the patients had had major gastric surgery, including subtotal gastrectomy, gastroenterostomy, and/or vagotomy. Additional cases of this type have recently been described by Black and associates.[17] In the present study (Table I) 9 of the 178 patients with gastroenteritis had had major gastric surgery for complicated duodenal ulcer, from a few weeks to as long as ten years prior to the onset of *Salmonella* enteritis. It is of interest that the series did not contain a single patient with unoperated peptic ulcer.

Altered susceptibility to enteric infection after major gastric surgery might be associated with relative or absolute achlorhydria, rapid emptying of gastric contents into the small intestine, secondary changes in normal intestinal flora or other factors.[15] It is known that normal gastric juice, with its high hydrogen ion concentration (pH 2-3), is bactericidal for a number of organisms that infect by the oral route,[18, 19] and Meynell[20] has demonstrated that *S. typhimurium* is killed almost instantaneously at pH 2 or below and somewhat more slowly at pH 2-3. It has also been shown that after subtotal gastrectomy the stomach functions as a widely patent tube which permits rapid emptying of its contents into the small intestine.[21] Since the development of enteritis depends on delivery of a certain number of viable bacilli to the small intestine, it is reasonable to assume that altered gastric acidity or emptying time might profoundly influence susceptibility to enteric infection.

Although the increased susceptibility of patients with previous gastric surgery to *Salmonella* gastroenteritis has been adequately documented, additional detailed studies are needed to define the frequency of infection, especially subclinical infection, and the precise mechanism

of the altered susceptibility. A similar relationship may exist between *Salmonella* enteritis and other diseases that alter gastric function. At least the occurrence of *Salmonella* gastroenteritis in two patients with extensive neoplastic involvement of the stomach and one patient with atrophic gastritis and achlorhydria (Table I) suggests that additional observations would be of interest.

Waddell and Kunz[15] noted that patients with *Salmonella* gastroenteritis after major gastric surgery were severely ill and one of their nine patients died. The observations of Bengtsson and associates[22] related to an epidemic of *S. typhimurium* infection that occurred in Sweden in 1953 are pertinent. Eleven of their 654 patients died and postmortem examination revealed polyposis and carcinoma of the stomach in one patient and old subtotal gastrectomy and gastroenterostomy in two additional patients. In the present series, one of the fatal cases of gastroenteritis gave a history of a stomach operation, but postmortem examination was not performed.

Salmonellosis in Patients with Hemoglobinopathies. Although sporadic case reports of *Salmonella* osteomyelitis in patients with sickle cell anemia had appeared in the literature since 1925,[23] it was not until 1951 that Hodges and Holt[24] and Lambotte-Legrand[25, 26] first called attention to the relation between these diseases. Since these original notes, additional publications[23, 27-32] have appeared from all parts of the world, establishing beyond question that the incidence of severe *Salmonella* infections, especially osteomyelitis, is increased in patients with sickle cell anemia. For example, in a study[23] in Georgia, three cases of *Salmonella* osteomyelitis and one case of *Salmonella* bacteremia were discovered among a total of 36 randomly selected patients with sickle cell anemia; osteomyelitis caused by other microorganisms was not encountered. Roberts and Hilburg[29] at the Los Angeles County Hospital found only three children with *Salmonella* bone infection among a total of 865 with osteomyelitis and all three of these patients had sickle cell anemia. The four instances of *Salmonella* osteomyelitis described by Hughes and Carroll[33] among 130 children with pyogenic osteomyelitis in Tennessee were in patients with sickle cell anemia. Finally, van Oye[32] in a recent article mentioned that he was aware of 65 additional cases of *Salmonella* osteomyelitis in patients with sickle cell anemia in the Belgian Congo.

Approximately 100 cases of sickle cell anemia and severe *Salmonella*

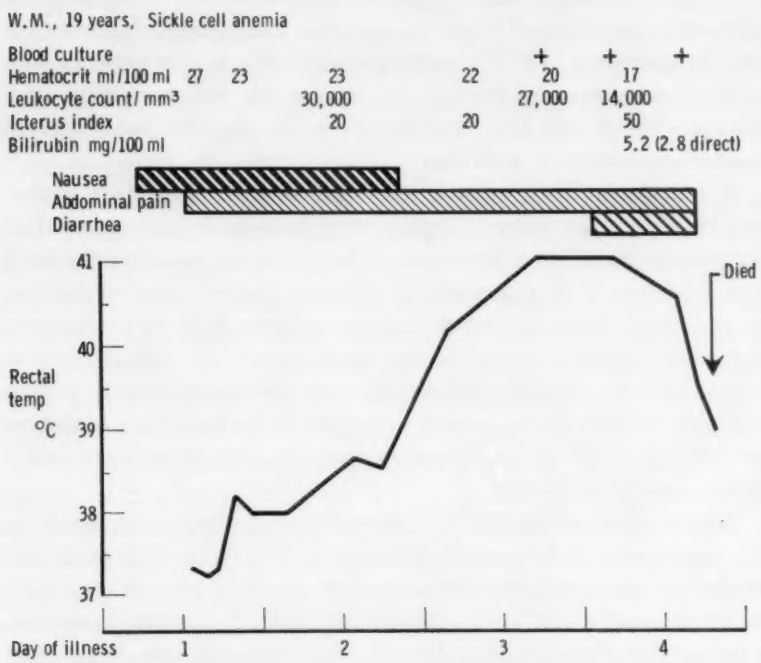

Figure 2—Course of illness in a 19-year old Negro male with sickle cell anemia and *S. choleraesuis bacteremia*. This patient had had numerous episodes of abdominal and bone pain since the age of one year. Three hours before admission the patient began to have nausea, cramping epigastric pain, chilly sensations and diffuse muscle pain. On admission to the hospital the leukocyte count was 30,000 cells per mm.^{3} and the hematocrit was 27 ml. per 100 ml. On the third and fourth days of illness he developed hyperpyrexia, diarrhea of moderate severity, and icterus. On the fourth day, the hematocrit was 17 ml. per 100 ml., hypotension appeared and the patient died. Blood cultures obtained during the last 36 hours of life subsequently yielded *S. choleraesuis*.

infection have been mentioned or thoroughly described in the literature; on the basis of these observations certain conclusions are warranted:

1. *Salmonella* bacteremia in patients with sickle cell anemia is usually associated with localization of infection in bone with the production of osteomyelitis. Only four exceptions to this generalization have been detected to date. Chernoff and Josephson[34] described a six-month old child with *S. choleraesuis* bacteremia without apparent bone localization, and Hook, Campbell, Weens and Cooper[23] studied a 23-year old man with documented sickle cell anemia and transient *S.*

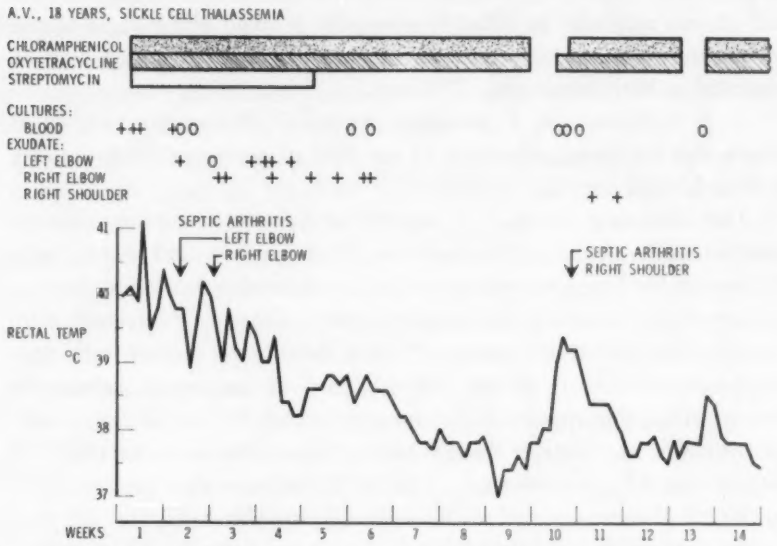

Figure 3—Course of illness in an 18-year old white female of Greek origin who had sickle cell-thalassemia and *Salmonella typhimurium* bacteremia, osteomyelitis, and arthritis. This patient had had many episodes of diffuse bone and abdominal pain since the age of eight years. Marked anemia was a constant finding. Splenectomy was performed at the age of 14 years. The present episode began with chills and fever; there were no symptoms of enteritis. On admission to the hospital numerous nucleated erythrocytes were present in the peripheral blood, the hematocrit was 18 ml. per 100 ml., the leukocyte count was 40,000 cells per mm.,³ and the total bilirubin was 2.9 mg. per 100 ml. Response to antimicrobial therapy was slow. Although pyogenic arthritis of the elbow joints developed during the second week of hospitalization, roentgenographic evidence of osteomyelitis of the radii, humeri, and ulnae was not detected until ten weeks after onset.

choleraesuis bacteremia. Two patients with sickle cell anemia in the present series died of overwhelming *Salmonella* bacteremia without evidence of localized suppurative infection (Figure 2).

2. *Salmonella* osteomyelitis in patients with sickle cell anemia occurs at multiple foci in approximately two-thirds of the cases. The long bones of the extremities are usually involved, but infection of metacarpals, metatarsals or phalanges has been reported in about one-fourth of the cases.

3. The recognition of pyogenic osteomyelitis in patients with sickle cell anemia is often difficult because fever and pain and swelling in the extremities, as well as leukocytosis, occur in both diseases. Roentgen-

ologic differentiation of early osteomyelitis and bone changes in sickle cell anemia may also be difficult, especially in small children, in whom soft-tissue swelling, periosteal reaction and cortical destruction may be observed in both conditions.

4. *S. typhimurium, S. paratyphi B,* and *S. choleraesuis* have been responsible for about two-thirds of the cases of osteomyelitis in patients with sickle cell anemia.

The increasing number of reports of *Salmonella* osteomyelitis in patients with sickle cell-hemoglobin C disease[29, 35] and sickle cell-thalassemia [32, 36] suggests strongly that persons with other hemoglobinopathies are also unusually susceptible to severe *Salmonella* infection with osteomyelitis. Silver and associates[36] have described a patient with cyanotic congenital heart disease, hemoglobin C-D disease and *Salmonella* osteomyelitis; this appears to be the only report to date of *Salmonella* osteomyelitis in a patient with a hemoglobinopathy not characterized by sickling. The present study (Table II) includes two patients with sickle cell-thalassemia and *Salmonella* osteomyelitis (Figure 3), one patient with sickle cell hemoglobin-C disease and *Salmonella* bacteremia, and one patient with sickle trait, hemolytic anemia, and *Salmonella* meningitis. Two patients with thalassemia major and bacteremia without apparent localization were also observed (Table II).

The mechanism of the altered susceptibility of patients with sickle cell anemia to systemic *Salmonella* infection is unknown. Ischemia or capillary thromboses may make the gastrointestinal tract less capable of resisting invasion by intestinal organisms, but at present there is no evidence to support this concept. It has been suggested that the frequent autosplenectomy in patients with sickle cell anemia may decrease resistance to infection[23] but at least one-third of the reported patients have had splenomegaly at the time of infection. McCormick, Flanigan and Francisco[37] have recently shown that there is no difference in the growth of *Salmonella* on the surface of blood agar plates prepared with normal or sickle cell blood. Additional studies indicate that sickle hemoglobin does not augment the growth of *Salmonella in vitro*,[38] and that there is no difference in bactericidal activity of sickle and normal hemoglobin.[39, 40] Impairment of antibody formulation in sickle cell anemia is unlikely as these patients show marked increases in antibody titer during the course of infection, and normal levels of gamma globulin.

The role of hemolytic anemia in the altered systemic susceptibility of patients with sickle cell disease to *Salmonella* infection remains to be defined. It is of interest to recall that malaria and bartonellosis, which are both characterized by hemolysis and anemia, also predispose to the development of *Salmonella* bacteremia.[3]

Studies[41] are in progress to determine the influence of anemia on the resistance of mice to infection with *Salmonella typhimurium*. The observation of Berry[42] that blood loss anemia does not increase susceptibility to *Salmonella* infection has been confirmed. However, in striking contrast, mice with hemolytic anemia induced with phenylhydrazine are impressively more susceptible to *Salmonella* infection than normal mice or those with blood loss anemia. Whether the alterations in susceptibility observed in this model are related to hemolysis or some other effect of phenylhydrazine is being investigated.

The reason for localization of *Salmonella* in bone of patients with sickle cell anemia or one of the sickle variants is also unknown, but the most logical explanation is that once *Salmonella* gain access to the blood stream they localize in areas of ischemia and necrosis which are so common in bones of patients with sickle cell disease. Numerous instances of localization of *Salmonella* in tissues that are the site of pre-existing disease have been recorded;[43] these include localization in bone compressed by aortic aneurysms, hematomas, areas of infarction, sites of intra-muscular injection of quinine hydrochloride, retention cysts of the kidney, corpus luteum and other ovarian cysts, uterine myomata, carcinoma, pheochromocytoma, the thyroid gland in exophthalmic goiter, subcutaneous nodules of onchocerciasis, echinococcus cyst of liver, and the urinary tracts of patients with urinary schistosomiasis. Localization of bacteria in diseased tissue is not an exclusive property of *Salmonella*, of course, and also occurs with staphylococci, pseudomonas and other organisms. Nevertheless, the extraordinary examples of localized *Salmonella* infections recorded in the literature suggests that a comparison of localization of *Salmonella* and other organisms in sites of pre-existing disease would be of interest.

Alterations in Susceptibility Induced by Antibiotics. It has been postulated frequently that the normal flora of the skin, mucous membranes and gut protect against invading pathogens, and that modification of this flora may increase susceptibility to bacterial invasion. One of the most impressive examples of altered susceptibility induced by

antibiotics has been offered by Miller and associates[44] who found that mice pretreated by the oral route with streptomycin were 100,000 times more susceptible to *S. typhimurium* inoculated intragastrically than saline-pretreated controls. This observation has been confirmed by Meynell.[20] Resistance of antibiotic pretreated mice was promptly restored by feeding fecal suspensions or fecal filtrates.[44]

The role of antibiotics in altering susceptibility of man to salmonellosis is unknown. Finger and Wood[45] in 1955 described a patient who developed *Salmonella* gastroenteritis while receiving prophylactic tetracycline therapy, and suggested a relationship between the antibiotic therapy and salmonellosis. Although five years have elapsed, additional cases of this type have not been described.

In the present series of 305 patients with salmonellosis a history of recent penicillin, streptomycin or tetracycline therapy was obtained from at least 15 patients. Antimicrobials were usually given because of "otitis", "pharyngitis", or "fever" shortly before onset of symptoms of gastroenteritis. It is clear that in certain cases the antibiotics were given for the intial symptoms of *Salmonella* infection, and in no case was the evidence convincing of a causal relationship between the antimicrobial therapy and the subsequent development of salmonellosis.

Summary

The outcome of the interaction of the human host and bacteria of the genus *Salmonella* is influenced by the dose of organisms ingested, the serotype of *Salmonella*, and the status of the host. In general, large numbers of organisms are required to produce disease in previously healthy persons, although with certain strains and in persons with certain underlying diseases, the dose required is probably much smaller.

Patients hospitalized with sporadic *Salmonella* infections often have a major underlying disease. Previous major gastric surgery predisposes to the development of *Salmonella* gastroenteritis, and sickle cell anemia and certain other hemoglobinopathies increase susceptibility to blood stream invasion and bone localization by *Salmonella*. The mechanisms by which these entities alter host resistance to *Salmonella* infection are poorly understood and many additional observations are needed.

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Advance Announcement

FIFTH ANNUAL POSTGRADUATE WEEK

October 23 to 27, 1961

SURVEY AND REVIEW

The physiology, diagnosis and therapy of selected aspects of a specific disease will be presented each day. The program will include panel discussions and individual papers.

Daily Sessions — 10 a.m. to 12 noon, and 1 p.m. to 3 p.m.
No evening sessions.

Monday	**GENETICS**
Tuesday	**Morning — RHEUMATOID ARTHRITIS** **Afternoon — SYSTEMIC LUPUS ERYTHEMATOSUS**
Wednesday	**Morning — EPIDEMIOLOGY OF CANCER** **Afternoon — BREAST CANCER**
Thursday	**EDEMA AND ANASARCA**
Friday	**LIVER DISEASE**

A subscription luncheon will be served in the Presidents' Gallery at the Academy each day.

REGISTRATION

Members of the medical profession, including interns and residents, and those working in the allied sciences, are cordially invited to attend all sessions. Registration, WITHOUT FEE, is required for non-Fellows of the Academy.

A detailed program, when published, will be mailed to every Fellow of the Academy without request and to others on request addressed to:

The Secretary, Postgraduate Week
The New York Academy of Medicine
2 East 103 Street, New York 29, N. Y.

THE LOUIS LIVINGSTON SEAMAN FUND

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The New York Academy of Medicine announces the availability of The Louis Livingston Seaman Fund for the furtherance of research in bacteriology and sanitary science. Four thousand seven hundred and ninety-five dollars is available for assignment in 1961. This Fund was made possible by the terms of the will of the late Dr. Louis Livingston Seaman and is administered by a committee of the Academy under the following conditions and regulations:

1. The Fund will be expended only in grants-in-aid for all or any part of the sum available for investigation, scholarships for or travel related to the furtherance of research in preventive medicine or public health.
2. Preference will be given to applications from medical students, interns, residents and junior faculty members whose project may foster their understanding of research, including the historical perspectives thereof.
3. Geographically, preference will be given to applicants from the New York metropolitan area.
4. Projects for which other major sources of research funds are not applicable will also be given preference.
5. The committee will receive applications either from institutions or individuals up to September 1, 1961.

Communications should be addressed to:

Dr. William C. Spring, Jr., Chairman
The Louis Livingston Seaman Fund
The New York Academy of Medicine
2 East 103 Street, New York 29, N. Y.

DEPARTMENT OF HOSPITALS
125 WORTH STREET
NEW YORK 13, N. Y.

May 23, 1961

To: Fellows of The New York Academy of Medicine

The medical manpower problems facing the municipal hospitals are of such magnitude that it is necessary for the Department of Hospitals to take a number of steps to insure that people will receive medical care.

Among the various steps being taken to fulfill the Department's responsibility is one new approach which involves additional cooperation of the medical profession, namely the recruitment of physicians to take care of patients on the various ward services where there are severe deficiencies of interns and residents. These physicians will be paid for time spent on the wards carrying out the functions of the service as defined by the director of each service. They will also be paid to be on call certain nights, weekends and holidays. Recruitment and nomination of such physicians is a responsibility of the medical boards of each of the municipal hospitals. Each medical board is submitting to the Department its estimated requirements for physicians. Money has already been appropriated by the Board of Estimate for the purpose. This is a necessary and important service which must be rendered by the medical profession and we ask your understanding, your support and your cooperation.

If you are interested in participating in this program, regularly or on a stand-by basis in the event of emergency, please advise the Department of Hospitals by addressing a letter or by telephoning to the Secretary of the Commissioner.

It is also urged that each physician make special efforts to arrange coverage when he is not available. When patients call two or three physicians and find none of them available, they go to the emergency rooms of either voluntary or municipal hospitals, thus compounding the difficulties facing these hospitals. Your cooperation in assuring that your patients are covered will do much to ease the burden of the hospitals.

The coming year is a crucial test of the ability of the medical profession and of voluntary and municipal hospitals to meet the needs of the public. We are confident that we can deal with the situation competently with your help.

Ray E. Trussell, M.D.
Commissioner

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FOR ADVANCED STUDY ABROAD—1962-63
The New York Academy of Medicine

In the current year the Committee on Medical Education will award *one* Scholarship grant in the amount of $4,800.00 for the *academic year 1962-63*.

This Scholarship was established by the donor to provide aid for advanced study abroad in a clinical subject in preparation for the practice of medicine or surgery in any of their branches. As stipulated by the donor of the fund, candidates must be *male* interns or residents in voluntary, municipal or Veterans Administration hospitals in New York City.

The course of study, the auspices under which it is to be taken, and the place or places where study is to be pursued, must receive the approval of the Committee. Applicants are favored who, from their records, show themselves capable of advanced work in clinical medicine. The Committee stipulates that the study be pursued abroad, preferably in Great Britain or in continental Europe.

Applications for this Scholarship must be submitted by **September 1, 1961.** Candidates may secure application forms by addressing:

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*Gamble, C.J.: Am. Pract. & Digest. Treat. *11*:852 (Oct.) 1960. See also Berberian, D.A., and Slighter, R.G.: J.A.M.A. *168*:2257 (Dec. 27) 1958; Kaufman, S.A.: Obst. and Gynec. *15*:401 (March) 1960; Warner, M.P.: J.Am.M. Women's A. *14*:412 (May) 1959.

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ANTIDEPRESSANTS			"CNS stimulants and anti-depressants, if given to anxious patients, will increase the anxiety...."	+
ELAVIL	"...this drug [ELAVIL] acted both as a tranquilizer and as an anti-depressant..."[2] Many physicians customarily treat anxious or depressed patients with a combination of an antidepressant and a tranquilizer. This is seldom necessary when prescribing ELAVIL because it has both antidepressant and anti-anxiety properties.			++

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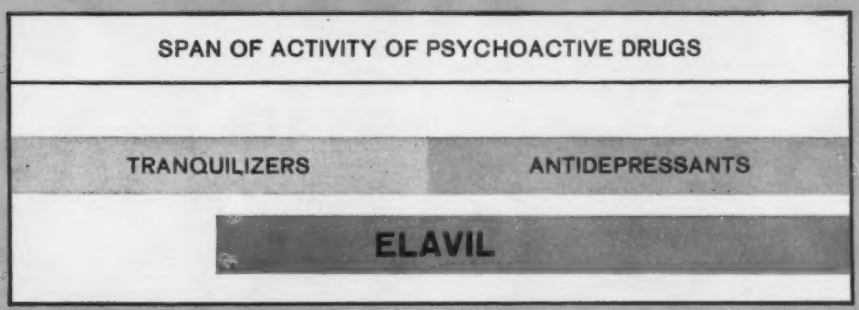

INDICATIONS: Manic-depressive reaction – depressed phase; involutional melancholia; reactive depression; schizoaffective depressions; neurotic depressive reaction; and these target symptoms: anxiety; depressed mood; insomnia; psychomotor retardation; functional somatic complaints; loss of interest; feelings of guilt; anorexia. May be used whether the emotional difficulty is a manifestation of neurosis or psychosis,[4] and in ambulatory or hospitalized patients.[3, 4, 5]

USUAL ADULT ORAL DOSAGE: Initial, 25 mg. three times a day, until a satisfactory response is noted. Many patients improve rapidly, although some depressed patients may require four to six weeks of therapy before obtaining maximum benefit. In severely depressed patients, as much as 150 mg. per day may be given. **Maintenance,** 25 mg. two to four times a day. Some patients may be maintained on 10 mg. four times a day. The natural course of depression is often many months in duration. Accordingly, it is appropriate to continue maintenance therapy for at least three months after the patient has achieved satisfactory improvement in order to lessen the possibility of relapse, which may occur if the patient's depressive cycle is not complete. In the event of relapse, therapy with ELAVIL may be reinstituted.

ELAVIL is not a monoamine oxidase (MAO) inhibitor. No evidence of drug-induced jaundice or agranulocytosis has been noted. Side effects (drowsiness, dizziness, nausea, excitement, hypotension, fine tremor, jitteriness, headache, heartburn, anorexia, increased perspiration, and skin rash), when they occur, are usually mild. However, as with all new therapeutic agents, careful observation of patients is recommended. As with other drugs possessing significant anticholinergic activity, ELAVIL is contraindicated in patients with glaucoma.

SUPPLY: Tablets, 10 mg. and 25 mg., in bottles of 100. **Injection** (intramuscular), 10 mg. per cc., 10-cc. vials.

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Before prescribing or administering ELAVIL, the physician should consult the detailed information on use accompanying the package or available on request.

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Riboflavin, mg.	0.6	50	Sodium, Gm.	0.2
Niacinamide, mg.	5	50	Potassium, Gm.	0.9
Calcium, Gm.	0.5	67	Copper, mg.	0.5
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references: (1) Nelson, W. E.: Textbook of Pediatrics, ed. 7, Philadelphia, W. B. Saunders Company, pp. 231-233, 1959. (2) Parrott, R. H., and Nelson, W. E.: *ibid.*, p. 759. (3) Johnston, J. A.: Ann. New York Acad. Sc. *69*:881-901 (Jan. 10) 1958. (4) Burke, B. S., and Kirkwood, S. B., in Greenhill, J. P.: Obstetrics, ed. 12, Philadelphia, W. B. Saunders Company, 1960, pp. 126-131. (5) Skillman, T. G.; Hamwi, G. J., and May, C.: Geriatrics *15*:464-472 (June) 1960.

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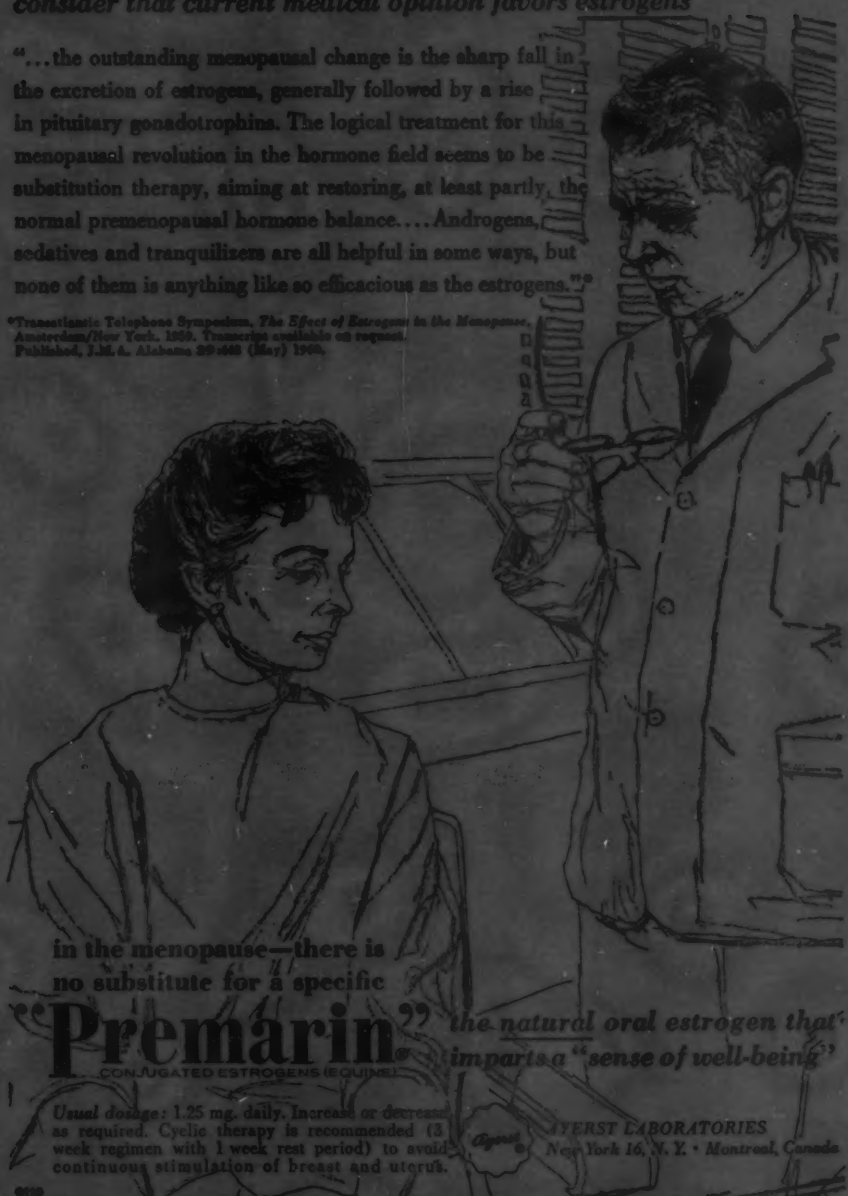
*Transatlantic Telephone Symposium, *The Effect of Estrogens in the Menopause*, Amsterdam/New York. 1959. Transcript available on request. Published, J.M.A. Alabama 29:448 (May) 1960.



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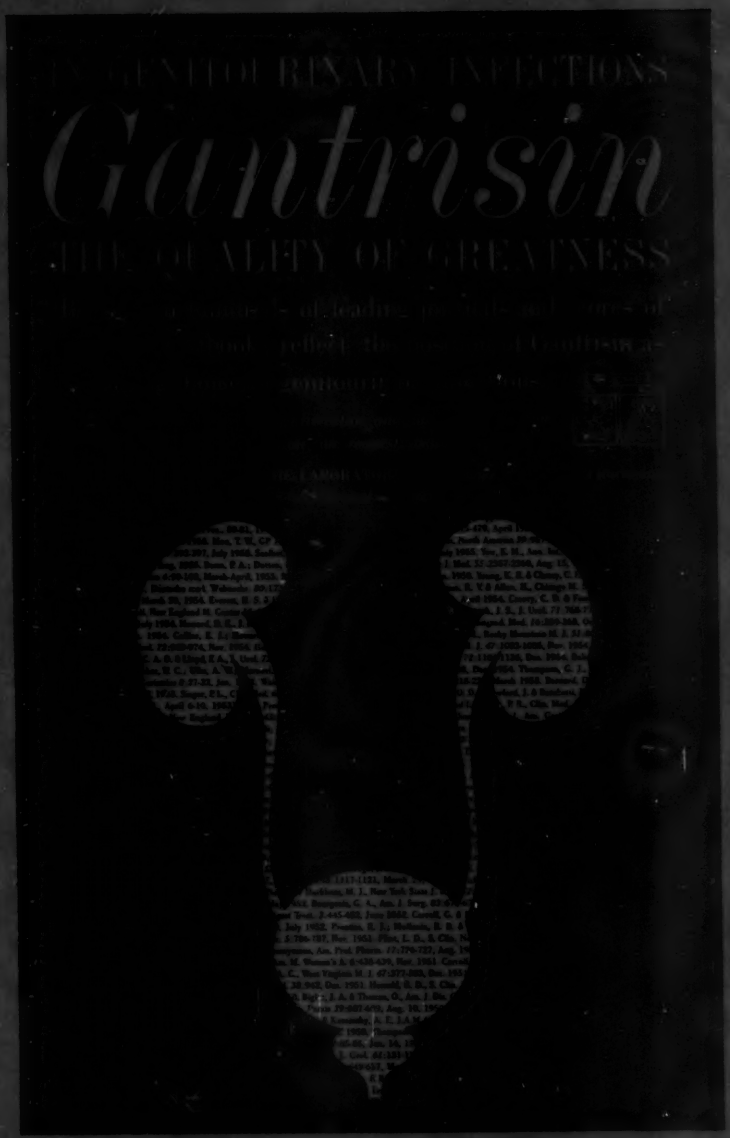
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